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Quantifying the Growth of Female Pacific Hake (*Merluccius productus*): An Example of Measuring Uncertainty and Bias in Non-linear Parameter Estimation

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Estimates of size-at-age are commonly reported in fisheries studies, but statistical uncertainty and intrinsic variability in the growth parameters are less frequently examined. We examine these questions using recently-developed statistical methods for quantifying uncertainty and bias in parameter estimates for nonlinear models with independent and additive Gaussian errors. We also describe diagnostic methods to determine when the usual method for examining parameter uncertainty, linearization, is unacceptable. Both our review and our approach to uncertainty are generally applicable to nonlinear estimation, and not solely restricted to growth models. We illustrate our approach with length-at-age data for female Pacific hake (*Merluccius productus*) from Georgia Strait, British Columbia. Between-year variation in somatic growth seems to be restricted to the two von Bertalanffy growth parameters K and L_∞. Mean asymptotic length declined by about 10% since the late 1970's while the parameter K nearly tripled in value. Closer examination showed that the decline in somatic growth was restricted to the maximum size attained. We argue that the changes are most consistent with selective removal of the largest individuals from the population by the fishery, rather than a response of growth rates to either environmental or density-dependent factors.

On parle souvent dans les études halieutiques d'estimation de la taille en fonction de l'âge, mais l'incertitude statistique et la variabilité intrinsèque des paramètres de la croissance sont moins souvent examinés. Nous étudions ces aspects à l'aide de méthodes statistiques récemment mises au point pour quantifier l'incertitude et le biais dans les estimations des paramètres pour des modèles non linéaires présentant des erreurs gaussiennes indépendantes et additives. Nous décrivons aussi des méthodes diagnostiques permettant de déterminer quand la linéarisation, méthode usuelle d'examen de l'incertitude des paramètres, est inacceptable. Notre synthèse ainsi que notre approche de l'incertitude s'appliquent de façon générale à l'estimation non linéaire, et ne se limitent pas aux modèles de la croissance. Nous illustrons notre démarche avec des données sur la longueur en fonction de l'âge chez des femelles de merlu du Pacifique (*Merluccius productus*) du détroit de Géorgie, en Colombie-Britannique. La variation annuelle de la croissance somatique semble limitée aux deux paramètres de croissance de von Bertalanffy K et L_∞. La longueur moyenne asymptotique a baissé d'environ 10 % depuis la fin des années 1970, tandis que le paramètre K a presque triplé de valeur. Une analyse plus approfondie a révélé que la réduction de la croissance somatique était limitée à la taille maximale atteinte. Nous soutenons que les modifications s'expliquent mieux par le prélèvement sélectif dû à la pêche des individus les plus gros d'une population que par une réponse des taux de croissance à des facteurs environnementaux ou dépendants de la densité.

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The growth of organisms reflects a complex and still poorly understood trade-off between somatic growth, reproduction, and the maintenance of basic physiological processes. It is clear that the trade-off between these competing demands is under evolutionary selection (Darwin 1958; p. 142-143; see also Alm 1959; and Stearns 1976). It is also frequently observed that many important life history processes, such as maturation, fertility, and (possibly) mortality are related to body size. Variation of somatic growth rate in response to selective pressures (for example, size-selective fisheries) is therefore likely. The possible causes of varying somatic growth rates are more varied however, and include a wide range of density-dependent and environmental factors.

Quantifying changes in somatic growth rates is not straightforward. First, given that a change may have occurred, does it reflect a change in the ultimate size attained by the animal, a change in the rate of somatic growth at some earlier point in the life history, or both? Second, how certain is it that one of the characteristics defining somatic growth has indeed changed, or can the observed differences reasonably be ascribed to sampling variation? And, finally, once clear-cut statistical evidence is obtained that somatic growth rates have indeed changed, what are the population consequences?

Variation in life history processes occurring outside the early life history period have generally been ignored in the search for the regulators of population productivity in marine fish. This paper examines the variation in somatic growth in Pacific hake (*Merluccius productus*). To do so, we address two questions. We first review several recently developed methods to examine

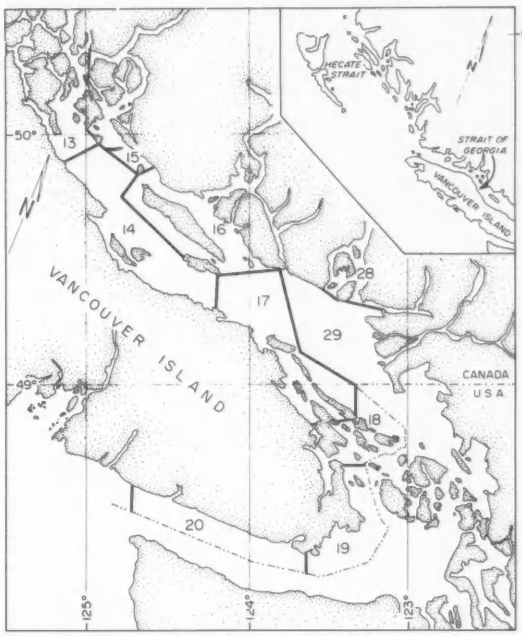

FIG. 1. Georgia Strait, British Columbia. The majority of samples used in the analyses were taken from Area 17; the remainder (ca. 10%) were taken from Area 16.

the reliability of the parameters estimated from growth models. Perhaps of more importance to the practising biologist, we illustrate a systematic method for identifying which aspects of somatic growth are variable. Our approach should be widely applicable to the study of variation in somatic growth rates, as well as to the wider problem of quantifying uncertainty in non-linear parameter estimates.

The use of these techniques allows us to determine which factors drive the observed variations in somatic growth. We illustrate our approach with an analysis of length-at-age data for immature and female Pacific hake in Georgia Strait, British Columbia. In subsequent papers we measure the changes in female maturity rates for this population and examine the inter-relationship between the changes in growth and maturity rates, and their possible relationship to density-dependent and environmental variables (D. W. Welch and G. A. McFarlane, unpubl. data)

Materials and Methods

Large concentrations of Pacific hake were discovered in the Strait of Georgia in 1974 (Westrheim 1974) and commercial exploitation started in 1978. Strait of Georgia hake can be distinguished from a large migratory stock restricted to the offshore region by their smaller asymptotic size and by the absence of the parasite, *Kudoa paniformis* (McFarlane and Beamish 1985). Much of the basic biology known about this stock is summarized in McFarlane and Beamish (1985).

The key data used in this paper are length-at-age measurements obtained from samples collected onboard research and commercial fishing vessels. We restricted our analysis to female and immature hake collected between January and April ($N = 2990$), with the bulk of the samples taken in February and March, just prior to the spawning season (March to May). Most samples come from trawl-caught hake taken from Statistical Area 17 of the Strait of Georgia (Fig. 1), but a few samples have also come from Area 16. Fork length was measured to the nearest 0.5 cm, and recorded along with other biological data (chiefly maturity state and sex). We rounded these lengths to the nearest centimetre and deleted a few (<20) anomalous records before the analysis began. Age was determined from otoliths using the break and burn method (Chilton and Beamish 1982).

It is impractical to distinguish the sex of immature hake (McFarlane and Beamish 1985), so males cannot be excluded from the sample of 1 and 2 yr-old fish. Inclusion of males could distort our results if males and females have different growth rates. However, the mean length of sexually mature 3 and 4 yr-old hake is virtually identical for the two sexes (McFarlane and Beamish 1985, figure 14), so it is reasonable to assume that a similar growth pattern holds for the immature fish. (Growth patterns for older fish do differ by sex, however). More importantly, immature fish, regardless of size, do not reproduce, and therefore do not directly influence the population productivity.

Time series of length-at-age data for 1 and 2 yr-old hake collected in the first quarter of 1975 and 1976 (McFarlane and Beamish 1985; figure 12) indicate that they grow ca. 3 cm between January and April, as contrasted with an annual growth of ca. 17 cm. We could not further restrict our selection criteria without significantly reducing sample sizes. However, our growth estimates (presented later) indicate that between-year variability in growth can be much greater than 3 cm, suggesting that year-to-year variation in the mean time samples were collected is not a major influence on our results.

Analytical Methods

Schnute's Model

Schnute (1981) proposed a flexible four-parameter model to describe somatic growth which includes most of the growth models historically used in fisheries research as submodels. In the general situation where the parameters a and b are non-zero, the model takes the form (Schnute 1981, equation (15)):

$$(1)\quad Y(t) = \left\{ y_1^b + (y_2^b - y_1^b)\,\frac{1 - e^{-a(t-t_1)}}{1 - e^{-a(t_2-t_1)}} \right\}^{1/b} \quad (a,b \neq 0),$$

where Y(t) is the estimated length at age t. Schnute (1981) gives limiting expressions for equation (1) when either or both the parameters (a,b) are zero. The four-parameter vector, $\Theta = (a,b,y_1,y_2)^T$, must be estimated from the data. As Schnute (1981) points out, these parameters have clear biological interpretations: y_1 and y_2 represent size at two ages t_1 and t_2, which are generally taken to be the ages of the youngest and oldest fish in the sample. The parameters a and b describe how the size-at-age curve connects these two ages.

Because Schnute's model encompasses a wide range of growth forms, it can be used to systematically assess the type of growth model best describing the data. For most (but not all) of the hake data sets we examine below, Schnute's model indicated that a von Bertalanffy-type growth model was appropriate, with estimates of the parameters $a,b>0$. However, in common with other growth models, the parameter estimates obtained from the model will usually be highly correlated. For example, nearly identical curves can be obtained by increasing

a and decreasing b (or vice versa) from their true values, and similar observations hold for other possible parameter pair combinations such as y_1 and a. This can lead to difficulty in interpreting the biology behind these curves, even when the parameters have well-defined meanings. For a similar result, see Gallucci and Quinn (1979).

In fact, during the initial stages of estimation when all four parameters were unconstrained, what appeared to be very different combinations of the parameters frequently gave essentially identical fits to the data. While this indicates that different parameter combinations provide equally plausible descriptions of the data, it is of little help to the biologist trying to establish what biological processes underlie the variability. It also suggests that for some purposes the model may be over-parameterized, and that once a growth form has been established, only a subset of the growth characteristics may actually vary between samples. We address the issue of determining which characteristics of growth may be changing in subsequent sections of this paper.

Von Bertalanffy's Model

Von Bertalanffy's model is appropriate for describing somatic growth when the rate of growth decreases linearly as some maximum size is approached. Von Bertalanffy's curve can be written as

$$(2)\quad Y(t) = L_\infty\,[1 - e^{-K(t-t_0)}].$$

The three-parameter vector, $\Theta = (L_\infty, K, t_0)^T$, must be estimated from the data, where L_∞ is the maximum size reached, t_0 the estimated age at zero size, and K the shape parameter determining how rapidly growth declines with age.

For $a,b>0$, Schnute's model corresponds to a generalized form of von Bertalanffy growth, and the von Bertalanffy parameters t_0 and L_∞ can be derived from estimates of (a,b) (see Schnute, 1981, p. 1131–2). However, as the two parameters (a,b) also define the shape of the curve between these two points, Schnute's curve is potentially more useful for describing the trajectory between the minimum and maximum sizes. It turns out that, for our data, this flexibility is of little benefit and is outweighed by certain undesirable properties that result from the nonlinear form of Schnute's model.

Estimation

Length-at-age t, $Y_i(t)$, is typically observed for a set of $i = 1,..,N$ fish. We assume in what follows that the error in ageing fish is negligible, and that the departure of individual length-at-age observations from the model are attributable to individual variation in growth and to errors in measuring and recording fish length. This assumption is reasonable for Strait of Georgia hake. Somatic growth is largely complete by age 4 or 5, so error in correctly ageing older fish will have little effect on estimates of their length. Age validation studies suggest that the error involved in estimating the age of younger fish is minor (McFarlane and Beamish 1985). When this assumption cannot be met, an errors-in-variables model (e.g. Kendall and Stuart 1979) is required.

Individual fish of a given age may, however, deviate significantly from an average growth curve. It is therefore necessary to make some assumption about this error distribution before estimating the parameters of the curve. The individual length frequency distributions shown later suggest that the residual distribution is normal with nearly constant additive variance. Under the assumption of independent identically distributed Gaussian error the sum of squared errors is proportional to the log-likelihood. Maximum likelihood parameter estimates can therefore be obtained by minimizing the sum of squared errors:

$$(3)\quad S(\Theta) = \sum_{i=1}^{N} [Y_i - Y_i(\Theta)]^2,$$

where N is the sample size, and $\Theta = (a,b,y_1,y_2)^T$ (Schnute) or $\Theta = (L_\infty, K, t_0)^T$ (von Bertalanffy) is the vector of parameters to be estimated.

Measures of Uncertainty

If growth models were linear it would be a simple matter to assess the uncertainty of parameter estimates in terms of confidence regions, and to use standard F-tests to determine if one or more parameters could be considered fixed between data sets. In fact, Schnute considers just this possibility, noting that "Even for non-linear models, the variance ratio (of competing models) is approximately F-distributed when sample sizes are large" (Schnute 1981; p. 1136).

Arguments based on (very) large sample properties of non-linear models are common, but questionable, as one usually does not know how large a sample is required. A related problem arises when comparing all or just a subset of the growth parameters. Not only are confidence intervals or regions based on a linearization of the original model about the parameter estimates unreliable (the procedure used in most texts and almost all statistical packages), the question of simultaneously comparing two alternative sets of several parameters can be quite complicated.

Likelihood Ratio Tests

The standard approach to this problem is to consider the joint distribution of the likelihood for the hypothesis H_0: $\Theta^* = \Theta_{ALT}$, where Θ_{ALT} is an alternative set of parameter values. Twice the difference in the log-likelihood obtained using these two alternative sets of parameter estimates may be compared with a χ^2_p distribution, where p is the difference in the number of parameters (Kendall and Stuart 1979, Chap. 24; Rao 1973, p. 417–418).

In the example we describe below, the alternative set of values are the parameters that best describe the aggregate data. In this case the null hypothesis of parameter equality over time may be tested by determining whether the test statistic exceeds the critical χ^2_p value. However, use of a Chi-squared statistic implies an infinite sample size, and therefore uses only the difference in the number of parameters in developing the test. We describe a more powerful test which takes into account sample size in the next section, which is applicable when the alternate set of parameter estimates can be considered "known" with negligible error. However, the conclusions which we subsequently draw are the same irrespective of which test we apply.

An alternative method to test for differences between sets of growth parameters is to use Hotelling's T^2 statistic (Bernard 1981). However, the results we report below suggest that when the level of nonlinearity in the sum of squares objective function is high, methods assuming that the objective surface is regular can seriously underestimate the uncertainty in probability statements. As the use of Hotelling's T^2 implicitly requires the use of these assumptions, we feel its use is questionable, for the reasons we subsequently discuss.

Confidence Regions

When the errors in a linear model are independent, normal, and identically distributed (nIID), the Gauss-Markov theorem guarantees that least squares estimation yields minimum variance unbiased parameter estimates (Kendall and Stuart 1979). When the model is nonlinear, there is no guarantee that either least squares or maximum likelihood parameter estimates are unbiased or minimum variance, and confidence regions based on a linearization of the model about the estimated parameters may be seriously in error.

Confidence regions and confidence intervals for nonlinear models are usually approximate. The most common method used to construct these regions (and the method used in essentially all commercial software packages) is based on a linearization of the model about the estimated parameters. In essence, linearization involves replacing the nonlinear model by a linear analogue at the maximum likelihood parameter estimates, and assuming that the confidence region constructed for the linear model is applicable to the nonlinear model as well. Whether these regions include the true parameter values with stated probability depends on the nonlinearity of the function being fitted, on the method used to construct confidence regions, and on the nominal confidence level, $1-\alpha$.

Numerical studies show that confidence regions based on the linearization method can have probability coverages as low as 12% for ostensible 95% confidence regions (Donaldson and Schnabel 1987). Their study also found that confidence regions constructed using likelihood methods consistently produced results that included the true parameter values with the correct probability. This result was consistent for a wide range of model functions, and even when sample sizes were small.

Likelihood-based confidence regions define a contour of constant likelihood over the objective surface, $S(\Theta)$. A confidence region constructed using the likelihood method is determined by finding those parameter values Θ that satisfy

$$(4)\quad S(\Theta) - S(\hat{\Theta}) \leq ps^2F_{p,N-p,1-\alpha},$$

where p is the number of parameters estimated, $\hat{\Theta}$ is the vector of maximum likelihood estimates, and $s^2 = S(\hat{\Theta})/(N-p)$ is the estimated variance (Donaldson and Schnabel 1987). From this definition, an alternative set of parameters Θ is considered statistically indistinguishable if they lie within this confidence region, and therefore have a relative log-likelihood within $ps^2F_{p,N-p,1-\alpha}$ of the maximum.

When the model is linear with nIID errors, this region corresponds exactly with the confidence region obtained by standard methods. We report a scaled version of statistic (4), denoted by an overbar, in the next section:

$$(5)\quad \bar{S}(\Theta) = \frac{S(\Theta) - S(\hat{\Theta})}{ps^2F_{p,N-p,1-\alpha}} .$$

Values of $S(\Theta)$ greater than 1.0 indicate that the alternative parameters lie outside the $100(1-\alpha)\%$ confidence region, and therefore that there is statistical evidence one or more of the component parameters of Θ do differ from the reference parameters.

Similarly, a confidence interval for an individual parameter such as L_∞ can be calculated from

$$(6)\quad S(L_\infty|\hat{\Theta}) - S(\hat{\Theta}) \leq s^2F_{1,N-1,1-\alpha},$$

but where $L_\infty|\hat{\Theta} = (L_\infty, \hat{k}, \hat{t}_0)^T$ now represents a vector in which only L_∞ is allowed to vary from the maximum likelihood estimate. Such confidence intervals can be interpreted as the projection of the confidence region onto the parameter axes (Donaldson and Schnabel 1987, p.71). Although we report them, we use them primarily as supplementary evidence to determine which biological characteristics of growth are least variable between data sets. This statistic is also reported as a scaled value; for example,

$$(7)\quad \bar{S}(L_\infty|\hat{\Theta}) = \frac{S(L_\infty|\hat{\Theta}) - S(\hat{\Theta})}{s^2F_{1,N-1,1-\alpha}} .$$

Diagnostic Statistics

Linearization methods may fail to yield accurate confidence regions for either of two reasons: the nonlinearity of the model function being fitted may be large, or the solution locus may not be close to planar for a sufficiently broad region about the estimated parameters. These two facets of nonlinearity may be diagnosed by decomposing the nonlinearity into effects due, respectively, to model parameterization, Γ_{PE}, and intrinsic nonlinearity of the coordinate grid, Γ_{IN} (Bates and Watts 1980). When these diagnostic statistics are less than

$$(8)\quad \Gamma_{IN} \leq 1 / 2\sqrt{F_{p,M-p,1-\alpha}}$$

and

$$(9)\quad \Gamma_{PE} \leq 1 / 2\sqrt{F_{p,M-p,1-\alpha}}$$

confidence statements based on the linearization method may be acceptable. Here, M is the number of different levels (i.e. ages) at which size is measured, and not the total number of size-at-age observations. Analytical formulae for these statistics are not available, but they can be quickly obtained by numerical methods. Fortran subroutines to do this are available in an appendix to Ratkowsky (1983), and a Pascal program based on this code is available from the authors of this paper.

In practice, all that is really desired is some assurance that the methods developed in linear statistical inference really are appropriate when applied to nonlinear situations. Numerical experiments support the conclusion that the Bates and Watts' statistics do provide a useful guide to the presence of unacceptably nonlinear behaviour in a wide variety of practical situations (Donaldson and Schnabel 1987), although examples have been found indicating that values smaller than the cut-off described above are required to ensure agreement (cf. Cook and Witmer 1985). In our study we report our confidence in the parameter estimates using the likelihood method. We report Bates and Watts' statistics primarily to illustrate that the commonly used linearization method seems to be inappropriate for establishing parameter uncertainty in nonlinear growth models.

Systematic departure of the estimated parameters from the unknown true values (bias) is also of concern, since there is no guarantee that the parameter estimates from nonlinear models are unbiased. We use Box's (1971) estimates of percent bias to predict which parameters may be inaccurately estimated. Nontechnical discussions of Box's estimate of bias and Bates and Watts' diagnostics are contained in Ratkowsky (1983); a more rigorous review is available in Bates and Watts (1988).

An alternative method for assessing bias and calculating confidence regions is the bootstrap (Efron 1982). We have used the methods described here because they have been shown to provide correct answers in a wide range of practical situations, and because they require much less effort than bootstrap methods.

TABLE 1. Sample sizes, N, and maximum likelihood (ML) parameter estimates $=\hat{\Theta}(\hat{L}_\infty,\hat{K},\hat{t}_0)^T$ for the length-at-age data sets shown in Fig. 2. The next two columns present the residual variance, $s^2=S(\hat{\Theta})/(N-p)$, and the scaled likelihood that the aggregate parameter estimates, $\tilde{S}(\hat{\Theta})$, lie within the 95% confidence region about the ML estimates. The last 3 columns give some idea which of the growth parameters differ from the aggregate estimates. Scaled likelihoods less than 1.0 (underlined) indicate that the aggregate estimates do not differ significantly from the maximum likelihood estimate for the individual data sets. Likelihood-based 95% confidence intervals for the aggregate estimates and those with t_0 constrained are shown in brackets.

						Scaled likelihoods			
	N	L_∞	K	t_0	s^2	$\tilde{S}(\hat{\Theta})$	$\tilde{S}(L_\infty\|\hat{\Theta})$	$\tilde{S}(K\|\hat{\Theta})$	$\tilde{S}(t_0\|\hat{\Theta})$
A. Aggregate ($p=3$)									
1975–88	2990	46.2	0.52	0.51	11.2				
		(46.1, 46.4)	(0.52, 0.53)	(0.49, 0.53)					
B. Unconstrained ($p=3$)									
1975	140	59.4	0.32	0.33	15.89	5.5	140.5	223.0	5.4
1976	303	46.5	0.65	0.82	7.44	7.7	0.8	30.8	6.2
1978	46	48.5	0.42	0.29	7.93	2.3	1.6	9.6	13.7
1979	98	48.3	0.51	0.57	11.40	2.1	6.4	0.3	0.4
1981	519	47.4	0.50	0.47	10.98	5.2	14.8	1.6	0.3
1982	317	45.7	0.56	0.53	5.62	0.7	3.9	3.8	0.1
1983	320	46.0	0.50	0.71	8.30	23.4	0.5	2.6	23.6
1984	159	46.0	0.43	−0.19	6.49	2.7	0.3	14.2	18.4
1985	233	44.5	0.55	0.38	14.13	3.6	11.7	0.8	1.1
1986	221	51.6	0.09	−11.18	12.21	23.2	83.7	636.8	2458.6
1987	127	44.1	0.84	1.23	6.42	3.5	19.6	98.2	20.4
1988	507	43.4	0.62	0.63	6.20	8.6	106.5	106.7	12.9
C. t_0 constrained ($p=2$)									
1975	140	56.1	0.37	0.51	16.03	7.7	87.6	84.5	—
		(55.0,57.1)	(0.36,0.39)	(0.41,0.57)					
1976	303	46.7	0.58	"	7.53	9.1	2.3	6.4	—
		(46.4,47.1)	(0.56,0.61)	(0.42,0.70)					
1978	46	45.5	0.63	"	8.94	1.8	0.2	3.7	—
		(43.7,47.3)	(0.57,0.68)	(0.44,0.54)					
1979	98	48.5	0.49	"	11.34	2.6	7.7	1.5	—
		(47.7,49.3)	(0.46,0.52)	(0.43,0.63)					
1981	519	47.4	0.51	"	10.97	6.6	13.5	0.6	—
		(47.1,47.7)	(0.49,0.53)	(0.42,0.57)					
1982	317	45.7	0.55	"	5.61	0.9	3.6	2.8	—
		(45.4,46.0)	(0.53,0.57)	(0.43,0.60)					
1983	320	47.0	0.43	"	8.52	28.1	3.3	68.0	—
		(46.6,47.5)	(0.43,0.44)	(0.48,0.58)					
1984	159	44.9	0.57	"	6.82	1.9	9.5	2.9	—
		(44.5,45.3)	(0.54,0.61)	(0.15,0.59)					
1985	233	44.2	0.60	"	14.12	4.5	15.0	4.5	—
		(43.7,44.7)	(0.56,0.64)	(0.35,0.61)					
1986	221	43.0	0.83	"	13.58	23.3	39.6	81.4	—
		(42.5,43.5)	(0.78,0.89)	(0.27,0.61)					
1987	127	44.7	0.61	"	7.03	2.3	9.1	9.3	—
		(44.2,45.2)	(0.58,0.65)	(0.40,0.76)					
1988	507	44.0	0.57	"	6.23	10.6	67.8	27.7	—
		(43.7,44.3)	(0.56,0.58)	(0.49,0.55)					
D. L_∞, t_0 constrained ($p=1$)									
1975	140	46.2	0.55	0.51	19.7	1.0	—	1.0	—
1976	303	"	0.61	"	7.6	12.9	—	12.9	—
1978	46	"	0.61	"	8.8	2.8	—	2.8	—
1979	98	"	0.55	"	12.8	0.6	—	0.6	—
1981	519	"	0.56	"	11.6	2.7	—	2.7	—
1982	317	"	0.53	"	5.7	0.0	—	0.0	—
1983	320	"	0.45	"	8.6	42.9	—	42.9	—
1984	159	"	0.50	"	7.1	1.1	—	1.1	—
1985	233	"	0.50	"	15.6	0.6	—	0.6	—
1986	221	"	0.64	"	17.3	16.0	—	16.0	—
1987	127	"	0.54	"	7.7	0.4	—	0.4	—
1988	507	"	0.51	"	6.8	3.6	—	3.6	—

Although the bootstrap has the advantage of being at least partially free of distributional assumptions, it is not always a trivial matter to correctly set up the resampling procedures required to use the method. One further advantage of confidence regions calculated from the likelihood method is that they easily incorporate information about parameter covariance which is difficult to assess using the bootstrap technique.

Results

Parameter Estimation

Statistical description of between-year variation in somatic growth is unlikely to involve all the parameters of a given growth model. It is not, however, obvious which parameters may vary over time. To address this question, we first aggregated all available length-at-age data and estimated the growth parameters for the aggregate data, $\hat{\Theta}$. Although we do not independently know what the true growth parameters are for hake, the aggregate data set is sufficiently large that there is little statistical uncertainty as to what range of parameters best describe it (Table 1. A). If there is no difference in the annual samples then the parameter estimates for the aggregate data should describe the annual data equally well.

Aggregate and annual length-at-age data sets are presented in Fig. 2. The body of each graph shows the length-frequency histograms for each age-group, with fish aged 10 yrs and older grouped in the last histogram and fish $\geq$60-cm fork length plotted at the 60-cm boundary. (Actual ages and lengths were used in all calculations). The histograms reveal little evidence of any significant departure from normality, so the assumption of nIID errors for this population seems reasonable.

Point estimates of the parameters for the annual data sets exhibit large between-year differences (Table 1.B). A test of whether the growth parameters do vary between years can be obtained by calculating the scaled relative likelihood (equation (5)) with the aggregate parameter estimates substituted for the maximum likelihood estimates, $\bar{S}(\hat{\Theta})$. In all years except 1982 (Table 1.B) the scaled likelihoods exceed 1.0, so the confidence regions about the annual length-at-age parameter estimates do not contain the aggregate estimates. The scaled relative likelihood calculated with each of the maximum likelihood estimates replaced in turn by the corresponding aggregate estimate also exceeds 1.0 in most cases.

There is therefore good evidence for between-year differences in somatic growth. However, in most years at least one growth characteristic does not show significant differences. Since there are very few observations of 1- and 2-yr-old fish in most years, the growth curve is poorly defined for these age groups. We therefore chose to constrain t_0 to its aggregate estimate, 0.51 cm (Table 1.A), and to refit the other parameters under this constraint. As female maturation does not begin until age 3 (McFarlane and Beamish, 1985), variation in the remaining two parameters still allows the curve to fit the data for the reproductively active age groups that are of primary interest.

In most cases, parameter estimates and residual variances change little after refitting (Table 1.C). The measures of scaled relative likelihood again indicate that parameter estimates for individual years are significantly different from aggregate estimates, with estimates of both L_∞ and K most at variance with the aggregate values.

There is some indication from the magnitude of the scaled likelihood $\bar{S}(L_\infty|\hat{\Theta})$ that asymptotic length varies between years. However, in most cases this difference is small, and the greatest discrepancy occurs in years with few large fish in the sample. As fork length is only recorded to the nearest cm and uncertainty in the aggregate estimate of L has been ignored in the calculation, it seems reasonable to examine the effect of fixing L_∞ at its aggregate value.

Sharp changes in the scaled likelihood and residual variance for some years after refitting with both t_0 and L_∞ constrained suggests that the curves may now be overconstrained (Table 1.D). In most cases the aggregate parameters still lie outside the joint confidence regions, however, and confidence intervals about K, the remaining parameter, do not include the corresponding aggregate estimate.

As a further approximate test of parameter equality, we calculated the difference in the residual variance between the constrained and unconstrained models, and then divided this value by the residual variance for the unconstrained model. We then compared these values with an F-statistic as suggested by Schnute (1981, p.1136). The results were roughly consistent with our previous analysis; only four of 12 comparisons between the unconstrained and t_0 constrained models were significantly different, while fully 10 of 12 possible comparisons between the $t_0 - L_\infty$ constrained model and the unconstrained model were significantly different. We therefore conclude that variation in at least the two parameters L_∞ and K is required to fully describe between-year differences in somatic growth for these data sets.

Effects of Nonlinearity: Γ_{PE}, Γ_{IN}, and Bias

As we have noted, parameter estimates for nonlinear models may depart from linearity for two reasons: the objective function $S(\Theta)$ may be insufficiently planar near the estimates (intrinsic nonlinearity), or the specific model function used may be sufficiently nonlinear that the projection of the parameter axes onto the objective surface may be unacceptably distorted (parameter nonlinearity).

The results (Table 2) indicate that Schnute's model is undesirably nonlinear when applied to the hake data, with both aspects of nonlinearity exceeding critical values, but that the von Bertalanffy model is likely to yield parameter estimates with nearly linear behaviour (unbiasedness, minimum variance, and accurate confidence regions). The predicted bias in the estimated parameters for von Bertalanffy's model support this conjecture, with estimates generally less than 1%. Estimated biases for the parameters of Schnute's model were usually (but not always) much larger.

Since the scaled parameter effects for von Bertalanffy's model consistently exceed 1, there is some evidence that an appropriate reparameterization of the model might lead to further reductions in bias (see Ratkowsky 1983). However, given the small bias evident, we did not pursue this possibility further.

Variation in Somatic Growth Over Time

The parameter estimates given in Table 1 show that mean asymptotic length rose to a maximum in 1979, and then fell sharply following the start of the commercial fishery (Fig. 3). Fluctuations in K (which determines how rapidly an initial growth rate declines with age) follow an inverse trend to that of L_∞. In most years when fluctuations in estimates of K did not closely follow those evident in the estimates of L_∞, sample sizes for young fish were small (indicated by arrows in Fig. 3; see also Fig. 2).

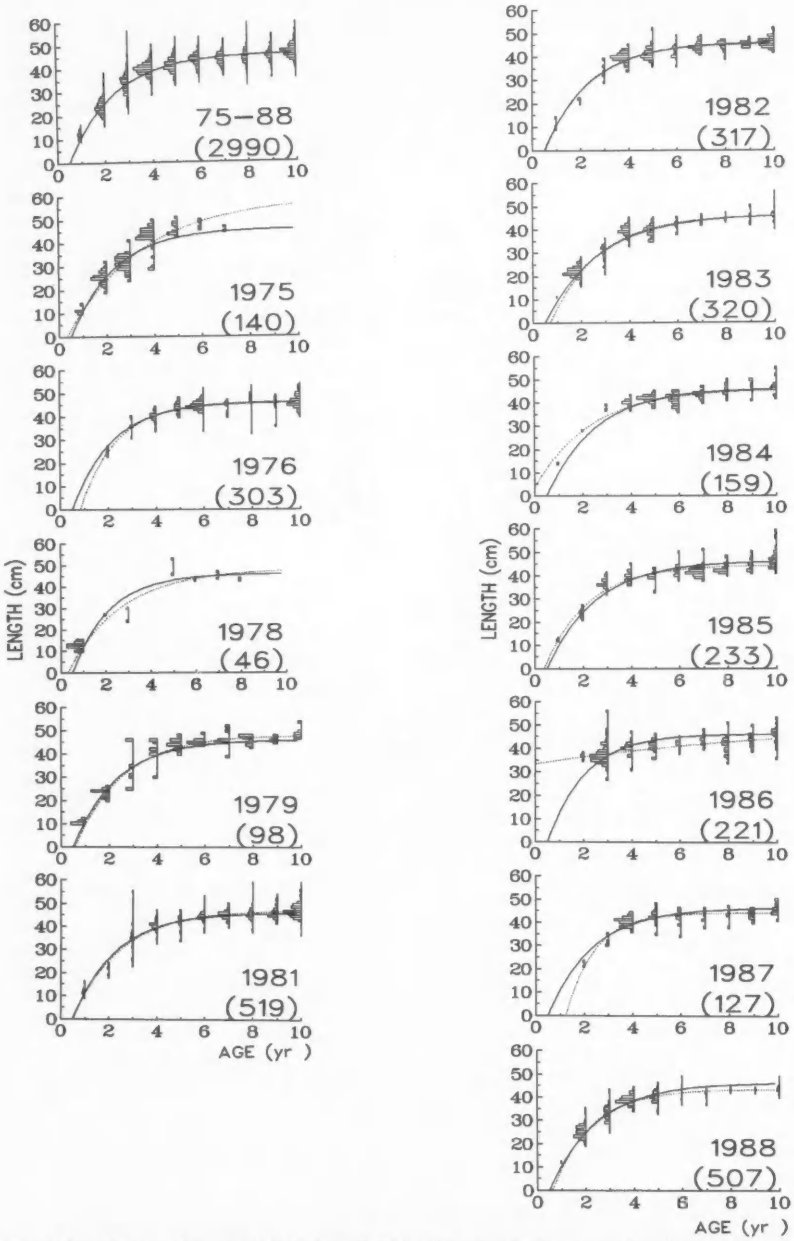

FIG. 2. Length-at-age data sets and fitted von Bertalanffy curves for the aggregate and annual data sets. Each graph shows the length-frequency distribution for individual age-groups, with observations for ages ≥10 yrs and lengths ≥60-cm plotted on the periphery. Unconstrained and constrained (L_∞, t_0) length-at-age curves are shown as dotted and solid lines, respectively. Sample sizes are in brackets.

Evidence that the growth parameters K and L_∞ tend to be inversely correlated is not restricted to hake (see Gallucci and Quinn 1979). However, given that the previous analysis indicated that both K and L needed to be unconstrained for reasonable parameter estimates to be obtained, it is of some interest to examine the growth rate of hake before maximum size is attained. The instantaneous growth rate is defined as the derivative of the von Bertalanffy growth curve (equation (2)) with respect to age, t:

$$(10) \quad Y'(t) = KL_\infty e^{-K(t-t_0)}$$

The maximum growth rate is attained at $t = t_0$, the age at size zero. By substituting into equation (10) estimates of K and L_∞ it is possible to estimate the somatic growth rate at ages prior

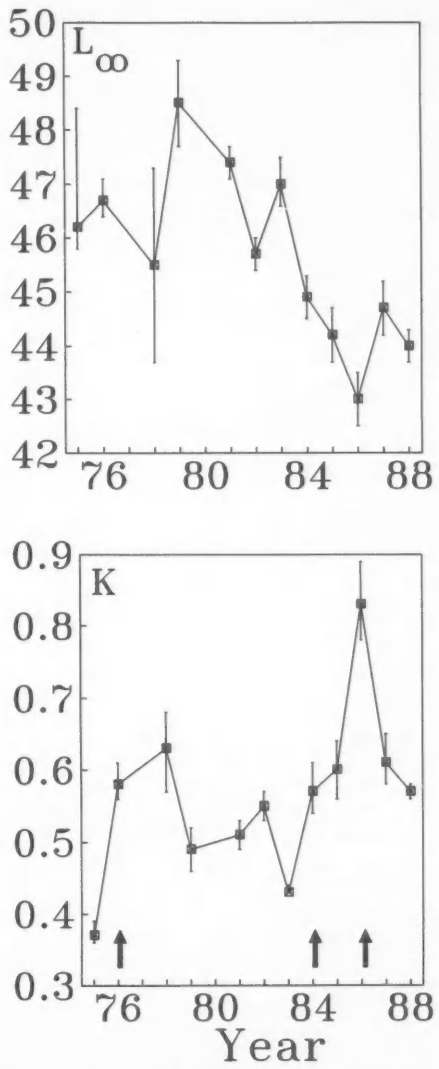

FIG. 3. Trends in the von Bertalanffy parameters L_∞ and K with time. The 95% confidence intervals on the parameter estimates are also shown. Arrows indicate years when sampling of young fish was particularly poor.

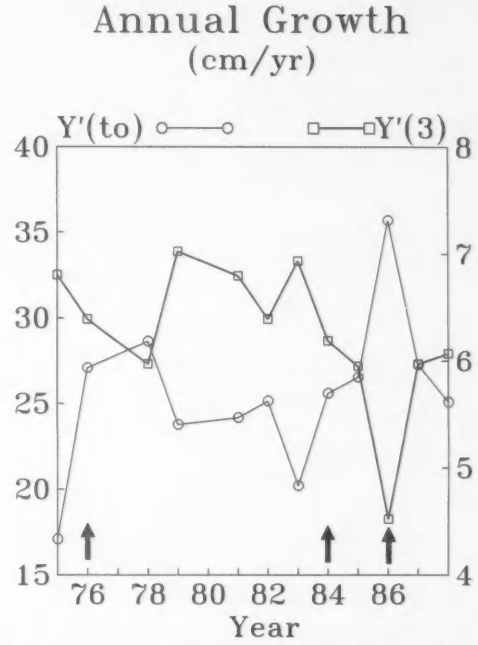

FIG. 4. Fluctuations in the estimated growth rate at ages t_0 and 3 yrs of age. Arrows indicate years when sampling of young fish was particularly poor. Although fluctuations in the growth rate are evident, there is only weak evidence of a trend in growth rate over time, in contrast to the trend for L_∞.

to maximum size being obtained. We calculated this growth rate at two ages: t_0 and $t = 3$ yrs of age, when maturation starts (Fig. 4). Perhaps reflecting the paucity of young fish in some of the samples, these growth estimates tend to be rather variable, but show only weak evidence of the marked decline evident in the L_∞ estimates. For example, ignoring the anomalous estimate in 1986, the growth rate at age 3 declined by at most 0.5 cm·yr^{-1} during the 1980's, and the pattern of fluctuation does not closely follow that evident in the estimates of L_∞. Of course, asymptotic size should more closely reflect events affecting the growth rates of the same cohort in years prior to when L_∞ is examined. However, given the limited data available, we did not pursue this possibility further.

TABLE 2. Comparison of the scaled measures of intrinsic nonlinearity, $\hat{\Gamma}_{IN}$, and parameter effects nonlinearity, $\hat{\Gamma}_{PE}$, for Schnute's and von Bertalanffy's models. Values >1 indicate possibly unacceptable levels of nonlinearity. The predicted bias (Box 1971) in the *ML* parameter estimates is shown as a percentage of the actual estimates for von Bertalanffy's model with L_∞, t_0 constrained. The magnitude of the estimated biases are similar for the unconstrained parameter estimates. In almost all cases estimates of the predicted bias in the parameter estimates for Schnute's model were greatly in excess of 100%. Software limitations on maximum array sizes prevented calculation of these measures for the 1981, 1988, and aggregate data sets.

	Schnute		von Bertalanffy (Constrained, p = 1)				
					Percent bias		
	$\hat{\Gamma}_{IN}$	$\hat{\Gamma}_{PE}$	$\hat{\Gamma}_{IN}$	$\hat{\Gamma}_{PE}$	$\hat{L}_\infty$	K^*	$\hat{t}_0$
1975	⩾100.0	⩾100.0	0.7	11.1	0.5	0.3	−2.0
1976	0.7	101.7	0.5	3.6	0.0	0.3	−1.7
1978	⩾100.0	⩾100.0	0.9	12.6	0.4	1.7	−1.0
1979	⩾100.0	⩾100.0	0.6	3.2	0.1	0.3	−0.9
1982	–no convergence–		0.2	1.4	0.0	0.0	−0.6
1983	4.3	12.8	0.3	3.8	0.1	0.2	−0.3
1984	1.2	28.6	0.5	3.9	0.1	0.2	−2.1
1985	0.3	14.9	0.5	3.3	0.1	0.1	−1.5
1986	4.5	⩾100.0	1.2	14.6	0.1	0.4	−9.1
1987	1.4	⩾100.0	0.7	7.6	0.1	0.1	−6.0

Discussion

Based on our study, we can draw conclusions about the utility of Schnute's and von Bertalanffy's growth models for examining variation in somatic growth rates in hake, and about what facets of model nonlinearity are of greatest importance for inference testing and parameter estimation in growth models.

We find that von Bertalanffy's model gives a more satisfactory description of length-at-age in female hake than does Schnute's (1981) general model, with essentially identical fits and nearly unbiased parameter estimates. Similar fits to the data are unsurprising, as Schnute's model includes the von Bertalanffy as a submodel. However, parameter estimates for Schnute's model indicated that an entirely different model, the sigmoid, provided the most likely description of somatic growth in some years when limited samples of young fish were available. Use of the scaled likelihood equation (5) allowed us to reject the possibility that growth in different years followed different functional forms, and to conclude that von Bertalanffy growth was the most appropriate descriptor for all data sets.

The ability of Schnute's model to describe different growth models is clearly one of its most attractive features, and its flexibility is useful in combination with the statistical tests we have described to initially establish an appropriate model form. However, for more detailed examination of which growth parameters vary between years we found the added flexibility of Schnute's model unnecessary. In addition, in several instances we easily found parameter estimates for the three-parameter von Bertalanffy model with slightly smaller residual variance than were initially found using Schnute's four-parameter model.

Our results therefore suggest that Schnute's model be used chiefly when a decision about the appropriate model form (e.g. sigmoid versus simple asymptotic growth) is needed, as Schnute originally proposed. However, both the nonlinearity of Schnute's model and the indicated bias of the parameter estimates suggests that the model be used with caution, and that once the appropriate model "shape" has been determined the limiting form (von Bertalanffy, Gompertz, etc.) be used instead. However, Schnute's model encompasses even the linear growth model, where the effects of nonlinearity on parameter estimates and their uncertainty are absent by definition. Schnute's model may therefore perform better when applied to other data sets than we have found for the hake data.

The levels of nonlinearity we measured indicate that confidence intervals and regions about the parameter estimates for growth models should always be based on likelihood statistics, and not on linearization techniques. (The latter is the standard method used in commercially available software.) Inferences based on the linearization method are often seriously in error when the level of nonlinearity exceeds critical values (Donaldson and Schnabel 1987), and the actual probability contained within calculated confidence regions may be much lower than the stated value. As the calculation of confidence regions using the likelihood technique basically involves comparing the sum of squares for two sets of alternative parameter estimates using equation (5), the use of the likelihood method is very simple in practice.

Almost without exception we found that one of the two measures of nonlinearity, Γ_{PE}, exceeded the critical level for both growth models examined. We therefore recommend the use of the scaled likelihood equation (5) for testing whether an alternative set of parameter estimates are significantly different from the measured values. This statistic is easy to calculate, since it involves only the sum of squared residuals for the alternative parameter sets, but its use does presume that the alternative estimates can be assumed known with negligible error. For our study this assumption is reasonable, because the sample size on which the aggregate parameter estimates is based is large. When this assumption is not reasonable, a likelihood ratio test is still possible but is less powerful because it assumes both sample sizes are infinite.

Conclusions

Our results demonstrate that it is possible to measure relatively small changes in somatic growth with considerable statistical certainty. However, although we are able to show that a significant decline in mean asymptotic size has occurred for female hake, we find little evidence that somatic growth rates at younger ages have also declined significantly. The drop in L_∞ following the development of the fishery therefore most likely reflects the selective removal of larger fish. Despite the negatively correlated changes between the parameters L_∞ and K, there is little evidence for a systematic drop in the growth rates of younger fish that could explain the decline in L_∞.

We conclude that the observed changes in somatic growth are unlikely to be a response to either the declining density of the population as a result of exploitation, or to genetic changes. The latter is to be expected, since there has been little time for the fishery to have selected against faster growing genotypes. The generation length of female hake is roughly 6.5 yr, so exploitation over the last decade is too short to have been a significant factor. Thus, although the fishery may well have a depressing effect on somatic growth rates by selecting against faster growing genotypes, we find little evidence that this has yet occurred. It will be interesting to examine the trend in somatic growth rate in the future, although it will be difficult to separate genetic effects from changes in population density.

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Estimation of Whole Pond Respiration Rate[1]

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Madenjian, C. P., G. L. Rogers, and A. W. Fast. 1990. Estimation of whole pond respiration rate. Can. J. Fish. Aquat. Sci. 47: 682–686.

A regression application of the whole pond respiration–diffusion (WPRD) model to overnight observations of dissolved oxygen concentration (DO) was introduced as a new method to estimate whole pond respiration rate. A new modification of the Welch procedure was also presented. These two techniques were compared with the tangent method for whole pond respiration rate estimation. The tangent method required fitting a quadratic curve to observed DO; and then calculating the slope of the tangent line to the curve at the time the curve reached 100% saturation of DO. When the quadratic was fitted to DO observed during both day and light, the tangent method often yielded inaccurate estimates of whole pond respiration rate. Estimates by the tangent method were sensitive to the time at which 100% saturation of DO occurred. The WPRD model regression and the modified Welch procedure were recommended to estimate whole pond respiration rate.

L'application sous forme d'une régression du modèle WPRD (diffusion-respiration à l'échelle de l'étang) à des observations faites au cours d'une nuit sur la concentration d'oxygène dissous (OD) est présentée comme nouvelle méthode permettant d'estimer le taux de respiration à l'échelle de l'étang. Nous présentons aussi une nouvelle modification de la procédure de Welch. Ces deux techniques sont comparées à la méthode de la tangente. Dans cette dernière méthode, il faut ajuster une courbe quadratique aux mesures de l'OD, puis calculer la pente de la tangente à la courbe au moment où la courbe atteint 100 % de la saturation en OD. Lorsque la courbe quadratique était ajustée aux mesures recueillies le jour et la nuit, la méthode de la tangente donnait souvent des estimations inexactes du taux de respiration à l'échelle de l'étang. Les estimations obtenues par cette méthode étaient sensibles au moment où la saturation en OD atteignait 100 %. L'application du modèle WPRD avec régression et la procédure de Welch modifiée sont recommandées pour l'estimation du taux de respiration à l'échelle de l'étang.

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Estimation of pond respiration rate is of interest to both ecologists and aquaculturists. Ecologically, determination of system respiration is important for bioenergetic studies. Odum (1969) has classified ecosystems based on the ratio of primary productivity to respiration and has formulated an outline of successional changes in ecosystem respiration rate.

Moreover, an estimate of whole pond respiration rate can be used to estimate respiration rate of a pond component that is otherwise difficult or expensive to measure directly. For example, direct measurement of sediment respiration rate using domes is a tedious process. The estimate of sediment respiration rate, generated by estimating whole pond respiration rate and then subtracting rates for the other pond components from the total, is easier to obtain (Madenjian et al. 1987a). The whole pond respiration rate estimates can also be used to estimate the oxygen transfer coefficient of aeration devices in ponds (Madenjian 1988). Knowledge of such oxygen transfer coefficients can aid farm pond managers in choosing the appropriate aeration devices for their ponds.

Sargent and Austin (1949); Odum and Odum (1955); Odum (1956); and Odum and Hoskin (1958) used diurnal DO changes in a body of flowing water (such as a stream or water over a reef) to estimate community respiration rate. Welch (1968) adapted this "free water" method (as opposed to bottle incubation methods) to estimate respiration rate in standing water communities. Using this method, the observed drop in DO during a time interval was a function of pond respiration and net diffusion of oxygen into the pond. Welch accounted for diffusion by using three different values of the oxygen transfer coefficient: a low value for calm conditions, a moderate value for moderately windy conditions, and a high value for strong wind conditions.

Fast et al. (1988) have introduced the tangent line method for estimating whole pond respiration. This method involved fitting a curve to observed DO, and then constructing a tangent line to the curve at the point of 100% saturation. Hence the method was usually limited to cases in which a pond becomes DO undersaturated during the night (this limitation would not apply to cases in which the fitted curve could be extrapolated to a 100% DO saturation value). The tangent line technique has been applied to data sets comprised of hourly DO observations over a 24-h period, but has not been applied to data sets comprised of only nighttime DO observations.

We shall: (1) suggest a modification of the Welch procedure, (2) introduce a new approach to pond respiration rate estimation, (3) review different versions of the tangent method to estimate whole pond respiration rate, and (4) compare estimates of whole pond respiration rate for the above-mentioned methods in a case study.

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Materials and Methods

Analytical Methods

We have modified the Welch procedure by refining the estimation of the oxygen transfer coefficient. We estimated the oxygen transfer coefficient, KL, as a function of wind speed based on the following relationship presented by Banks and Herrera (1977):

$$(1)\quad KL = 0.0036(8.43W^{0.5} - 3.67W + 0.43W^2)$$

where KL is in metres per hour units, and W is the wind speed ($m \cdot s^{-1}$). To estimate respiration rate for a particular time interval i, the following model was fit through the two points that demarcate the interval:

$$(2)\quad \frac{d\,(DO)}{dt} = -RR_i + \frac{KL_i}{PD}\,(SAT(T,S) - DO)$$

where $\frac{d(DO)}{dt}$ = rate of change in pond DO with time (mg $O_2 \cdot L^{-1} \cdot h^{-1}$), RR_i = whole pond respiration rate in time interval (mg $O_2 \cdot L^{-1} \cdot h^{-1}$), KL_i = the oxygen transfer coefficient during time interval ($m \cdot h^{-1}$), PD = pond depth (m), DO = dissolved oxygen concentration (mg $O_2 \cdot L^{-1}$), and $SAT(T,S)$ = the equilibrium saturation level of DO (mg $O_2 \cdot L^{-1}$) in water of temperature T (°C) and salinity S ($g \cdot kg^{-1}$). The pond respiration rate, RR_i, was assumed to be constant during the time interval. Temperature was assumed to be linear with time during the interval. The oxygen transfer coefficient, KL_i, was determined from an estimate of wind speed for interval i. The drop in DO due to respiration during the time interval was equal to the pond respiration rate, RR_i, multiplied by the time duration of the interval. The estimate of whole pond respiration rate was equal to the sum of the drops in DO due to respiration for all of the intervals divided by the time spanned between the first and last DO observations. We shall call the approach illustrated by equation (2) the exact interval method.

To avoid numerical solution of a differential equation, equation (2) was approximated by:

$$(3)\quad \Delta R_i = \Delta DO_i + \frac{KL_i}{PD}\,(SAT(T_i,S) - DO_i)$$

where ΔDO_i = drop in DO (mg $O_2 \cdot L^{-1}$) during time interval i, ΔR_i = drop in DO (mg $O_2 \cdot L^{-1}$) during time interval i due to respiration, KL_i = oxygen transfer coefficient ($m \cdot h^{-1}$) for interval i, PD = average pond depth (m), DO_i = average of the two DO observations (mg $O_2 \cdot L^{-1}$) that define interval i, $SAT(T_i,S)$ = equilibrium saturation DO level (mg $O_2 \cdot L^{-1}$) for water temperature T_i (°C) and salinity S ($g \cdot kg^{-1}$), and T_i = average of the two water temperature (°C) observations for interval i. The drops in DO due to respiration, the ΔR_i's, are summed for all intervals and divided by the time elapsed between the first and last observations to estimate whole pond respiration rate. We shall refer to the method illustrated by equation (3) as the approximate interval method. The approximate interval method, like the exact interval method, represented a modification of the Welch procedure because the oxygen transfer coefficient was estimated using equation (1).

In this paper we introduce a new method to estimate whole pond respiration rate: the nonlinear least squares fit of the WPRD model (Madenjian et al. 1987b, 1988) to overnight DO observations (Fig. 1). The WPRD model was described by the following equation:

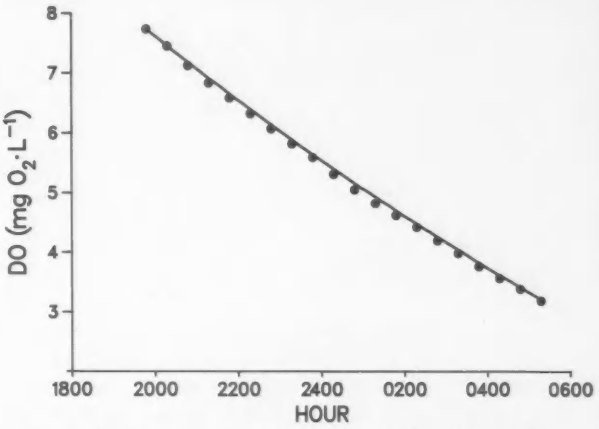

FIG. 1. Nonlinear least squares fit (solid line) of the WPRD model to observed DO (solid circles).

$$(4)\quad \frac{d(DO)}{dt} = ce^{kT} + \frac{KL}{PD}\,(SAT(T,S) - DO)$$

where c = respiration coefficient (mg $O_2 \cdot L^{-1} \cdot h^{-1}$), k = instantaneous rate of increase of respiration with temperature ($°C^{-1}$), T = water temperature (°C), and the other terms in the equation are defined as above. The oxygen transfer coefficient, KL, used in the model was based on an estimate of the average wind speed at night using equation (1). The initial boundary condition for the differential equation was that the DO of the fitted curve at the time of the first observation was equal to the observed DO at that time. The secant method of nonlinear least squares procedures was used (Ralston and Jennrich 1978).

The nighttime temperature curve was determined by a linear least squares fit of the following relationship:

$$(5)\quad T = ae^{-bt}$$

where T = water temperature (°C), a and b are constants, and t = time (h). The nighttime decline in pond temperature was adequately described by an exponential decrease (Madenjian et al. 1987b). Whole pond respiration rate was dependent on water temperature as follows:

$$(6)\quad RR = ce^{kT}$$

where RR = whole pond respiration rate (mg $O_2 \cdot L^{-1} \cdot h^{-1}$), and c and k are defined as above. The value of k was set equal to 0.0542 $°C^{-1}$ (Madenjian et al. 1987b).

The nonlinear least squares fit of the WPRD model yielded an estimate of the respiration coefficient. Then the whole pond respiration component of the WPRD model was integrated for the time course of the night, using the respiration coefficient estimate, to determine the nighttime drop in DO due to respiration. Nighttime decrease in DO due to respiration divided by the elapsed time between the first and last DO observation of the night yielded the average whole pond respiration rate. An assumption of the WPRD model was that the respiration coefficient remained constant through the night.

In its original application (Fast et al. 1988), the tangent line method was applied to DO observations for a 24-h period, from dusk to dusk (Fig. 2A). A quadratic was fitted to the DO observations. The point of 100% DO saturation on the

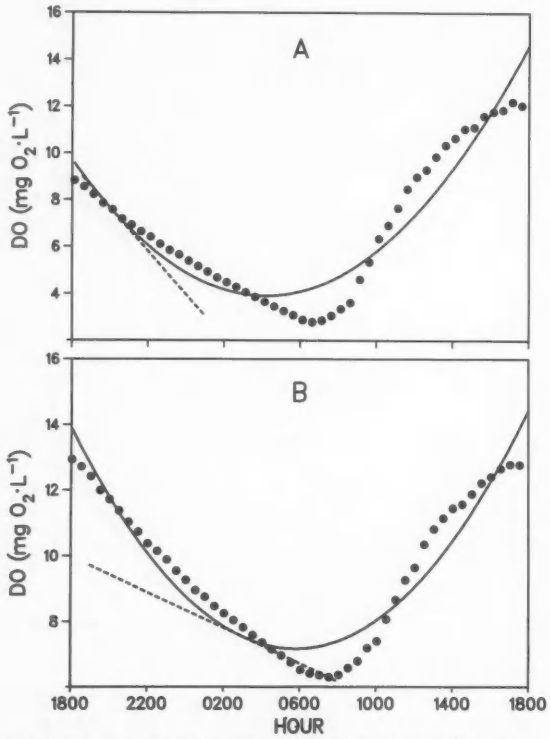

FIG. 2. Tangent method to estimate whole pond respiration rate. A quadratic was fit, by least squares, to observed DO (solid circles). The slope of the tangent (dashed line) to the fitted quadratic (solid line) at the time of 100% DO saturation yields an estimate of whole pond respiration rate. (A) Data for MRTC pond 9 beginning on April 28, 1987. (B) Data for MRTC pond 6 beginning on May 5, 1987.

descending limb of the fitted parabola was found and the magnitude of the slope of the tangent line to the parabola at 100% saturation was used as the estimate of whole pond respiration rate. At 100% DO saturation there was no net diffusion of oxygen between the pond and the atmosphere; the rate of change of pond DO at 100% saturation reflected solely on the whole pond respiration rate. Using the tangent line method, diffusion need not be taken into account in estimating whole pond respiration rate. Tangent method estimates of whole pond respiration rate were adjusted for water temperature effects using equations (5) and (6) (in the same manner that estimates from the nonlinear least squares fit of the WPRD model were adjusted).

A Case Study

An automated data logging system was established at the Mariculture Research and Training Center (MRTC) in Hakipuu (12 km north of Kaneohe on Oahu). DO and water temperature at middepth were monitored in four 0.2-ha ponds; in each pond, DO and water temperature were measured every 10 min for two grow-out periods (August through December 1986 and March through June 1987). The grow-out period referred to the period between stocking the postlarval shrimp (*Penaeus vannamei* and *P. monodon* were the cultivated species at the MRTC) into the pond and the shrimp harvest. Additionally, wind speed was measured every 10 s and then averaged every 2.5 min. Water was pumped from one fixed location in each pond (5 to 10 m from bank of pond) into a sampling chamber inside a shelter located at the center of the four-pond complex (see Madenjian et al. 1988 for more details).

TABLE 1. Estimates of whole pond respiration rate by various methods. *n* is the number of trials. Difference (diff.) refers to the estimate of whole pond respiration rate by a particular method minus the whole pond respiration estimate by the interval method. Min = minimum, Max = maximum. Data for shrimps ponds at MRTC in Hakipuu, Hawaii during August 1986 through June 1987.

		Respiration rate in mg $O_2 \cdot L^{-1} \cdot h^{-1}$			
Method	*n*	Mean WPR	Mean diff.	Min diff.	Max diff.
Interval	60	0.6253	0	0	0
Nonlinear least squares fit of WPRD model	60	0.6365	0.0112	−0.0913	0.2200
24-h tangent	54	0.7127	0.0690	−0.4166	0.3607
17-h tangent	57	0.8430	0.2062	−0.4439	0.6170
Night only tangent	60	0.6674	0.0421	−0.1348	0.5783

All of the above-mentioned methods to estimate whole pond respiration rate were employed on the same data set: 60 trials of nighttime data. For each trial, the data set was comprised of DO observations beginning approximately one-half hour after nightfall and ending approximately one-half hour before first daylight, in 10-min intervals. We expanded the data set for each trial to include data from dusk to dusk (a 24-h set) and we applied the tangent method to these 24-h data sets. We also evaluated the tangent method for trials which began approximately one-half hour after darkness and ended at noon the following day (approximately 17-h set).

Results

The exact interval method and the approximate interval method yielded the same estimates of whole pond respiration rate (within 0.0001 mg $O_2 \cdot L^{-1} \cdot h^{-1}$) for the 60 trials at the MRTC ponds. We shall hereafter group these methods and refer to either one or both as the interval method. The estimate of whole pond respiration rate from the nonlinear least squares fit of the WPRD model was not appreciably different from the estimate provided by the interval method (Table 1; Fig. 3). The absolute value of the difference between the two methods did not exceed 0.1 mg $O_2 \cdot L^{-1} \cdot h^{-1}$ for all 60 trials, except for one case in which the nonlinear least squares fit estimate was 0.22 greater than the interval method estimate.

In contrast, the tangent method estimates, particularly for the 24-h and 17-h versions, were considerably greater than the whole pond respiration estimate from the interval method (Table 1; Fig. 3). Although the mean whole pond respiration rate by the tangent methods was greater than that for the interval method, there were a few cases in which the whole pond respiration rate estimate by the tangent method was substantially less than that for the interval estimate.

For those tangent methods that involved analysis of daytime DO observations (17-h and 24-h methods), when the point of 100% DO saturation was reached before 02:00 h, the tangent method estimate was substantially greater than the estimate from the interval method (Table 2). On the night beginning on April 28, 1987, the point of 100% saturation occurred at 21:45 h (Fig.

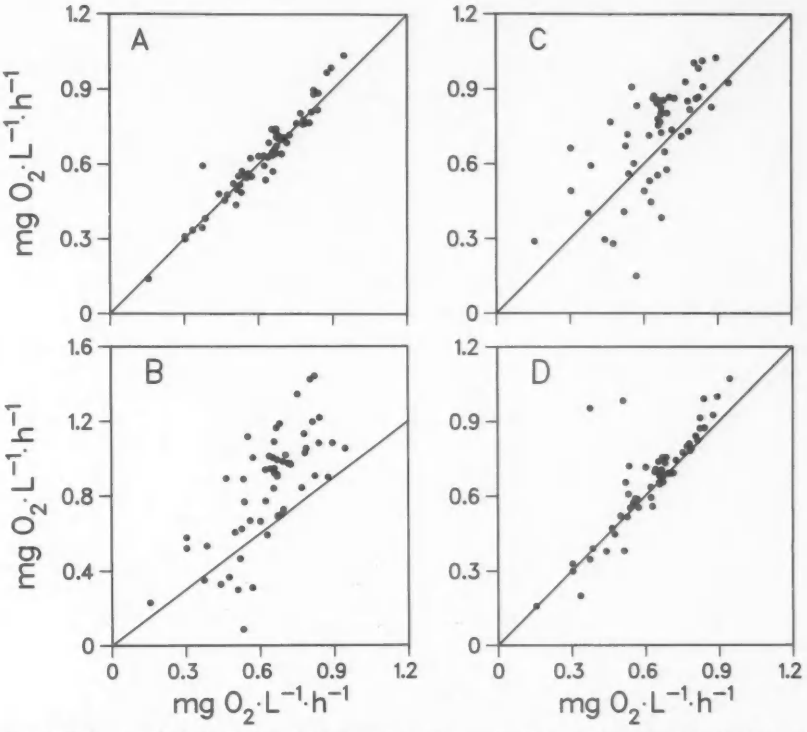

FIG. 3. Estimates of whole pond respiration rate by various methods versus the estimate by the interval method. x-axis variable is the pond respiration rate estimate by the interval method. (A) y-axis variable is pond respiration rate estimate by the nonlinear least squares fit of the WPRD model. (B) y-axis variable is 17-h tangent method estimate. (C) y-axis variable is 24-h tangent method estimate. (D) y-axis variable is night only tangent method estimate. Solid line is the locus of points at which the estimates by both techniques are equal.

TABLE 2. Estimates of mean whole pond respiration rate (mean WPR) by tangent methods for various times of 100% DO saturation. *n* is the number of trials. Difference (diff.) refers to the estimate of whole pond respiration rate by the tangent method minus the whole pond respiration rate estimate by the interval method. Data for shrimp ponds at MRTC in Hakipuu, Hawaii during August 1986 through June 1987.

			Respiration rate in mg $O_2 \cdot L^{-1} \cdot h^{-1}$	
Tangent method	Time of 100% DO saturation	*n*	Mean WPR	Mean diff.
24-h	After 0200 h	5	0.3042	−0.2307
24-h	Before 0200 h	49	0.7544	0.0996
24-h	Before 2400 h	37	0.7851	0.1379
24-h	Before 2200 h	13	0.7921	0.2182
17-h	After 0200 h	6	0.3100	−0.1971
17-h	Before 0200 h	51	0.9057	0.2536
17-h	Before 2400 h	36	0.9827	0.3352
17-h	Before 2200 h	10	0.8817	0.3527
Night only	After 0200 h	15	0.5844	0.0695
Night only	Before 0200 h	45	0.6951	0.0330
Night only	Before 2400 h	29	0.6913	0.0406
Night only	Before 2200 h	10	0.5561	0.0271

4A). Notice that the slope tangent line to the quadratic curve at this saturation point (-1.13 mg $O_2 \cdot L^{-1} \cdot h^{-1}$) was considerably steeper than the slope of the observed DO in the immediate vicinity of the 100% saturation point (approximately -0.6 mg $O_2 \cdot L^{-1} \cdot h^{-1}$). After 02:00 h, the slope of the tangent line to the quadratic was less than the slope of the observed DO curve.

For the 100% saturation event occurring after 02:00 h, both the 24-h and 17-h tangent method estimates of whole pond respiration were less than the interval method estimate. There were only five trials in which the point of 100% saturation occurred after 02:00 (for the 24-h tangent method) (Table 2). In these cases, the slope of the tangent line at 100% DO saturation was not as steep as the observed decline in DO (Fig. 2B).

When the quadratic was fit to night data only, the tangent method yielded higher estimates of whole pond respiration than by the interval method (Table 1); although the night only tangent method estimates were considerably closer to the interval method estimates than either of the 17-h or 24-h tangent method estimates (Fig. 3). When the tangent method was applied to night only data, there were a few cases in which the fitted quadratic curve had to be extrapolated well into the morning to reach the point of 100% DO saturation (Fig. 4B). Although the quadratic fit the observed data well during the night, the extrapolation of the curve appeared to result in underestimation of the whole pond respiration rate.

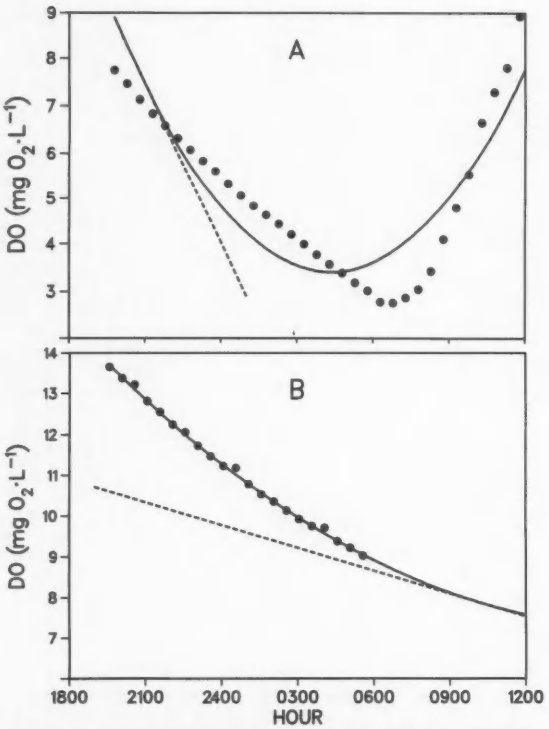

FIG. 4. The tangent method applied to observed DO (solid circles). The slope of the tangent (dashed line) to the quadratic (solid line) at 100% DO saturation yields an estimate of whole pond respiration rate. (A) Data for MRTC Pond 9 beginning on April 28, 1987. (B) Data for Pond 9 beginning on April 7, 1987.

Discussion

The nonlinear least squares fit of the WPRD model to the nighttime DO data yielded an estimate of whole pond respiration rate that was not appreciably different than the estimate by the interval method. This result indicated that: (1) the respiration coefficient did not substantially change during the night, or (2) the estimate of pond respiration rate by fitting the WPRD model to overnight DO data was robust to a dynamic respiration coefficient. The fact that the WPRD model performed well in predicting early morning DO, as shown by Madenjian et al. (1987b, 1988), supported the first alternative.

The tangent method for estimating whole pond respiration rate, in which the curve was fit to both daytime and nighttime DO data, was not recommended because the 24-h pond DO curve was asymmetric and was not satisfactorily modeled by a quadratic function. Tangent method estimates were sensitive to the time of 100% DO saturation. The earlier the time of 100% saturation occurred on the fitted curve, the greater the degree of overestimation of the whole pond respiration rate; the later the time of 100% saturation was reached, the greater the degree of underestimation of the whole pond respiration rate.

Our results suggested that the tangent method applied to night only data, on the average, slightly overestimated whole pond respiration rate. Moreover, if the time of 100% DO saturation had to be extrapolated into the morning, the whole pond respiration rate may be underestimated.

We recommended the interval method and the nonlinear least squares fitting of the WPRD model to estimate whole pond respiration rate. Because of asymmetry in the 24-h DO curve, tangent methods were not recommended when the quadratic regression curve was fitted to both daytime and nighttime DO data. The tangent method applied to night only data appeared to slightly overestimate whole pond respiration rate and should be used with caution, particularly when the fitted curve has to be extrapolated into the morning to reach 100% DO saturation.

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Lake Morphometry and Light in the Surface Layer

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Mean irradiance in the surface layer was calculated as a percent of incident irradiance using Secchi depths and thermocline depths for two sets of lakes: (1) 20 nearly circular lakes in northwest Minnesota, USA, sampled at a similar season, and (2) 1140 lakes of heterogeneous shapes in Ontario, Canada, sampled throughout the ice-free season. For both, as lake area increased, the thickness of the surface layer increased. Consequently, lakes larger in area had lower mean irradiance in their surface layer. Lake depth had less influence, but in the Ontario lakes, as depth increased, attenuation decreased, and mean irradiance increased. Lake area (log transformed) explained ca. one-half of the variation in mean irradiance in the homogeneous sampling of Minnesota lakes, while area and depth together explained less of the variation in the heterogeneous sampling of Ontario lakes. Shape, i.e. shoreline development, was unimportant compared with the gross measures of size, area and depth. Mean irradiance is complexly related to lake size; when depth increases slowly with area, larger lakes may have lower mean irradiance in their surface layers. But, when depth increases quickly with area, mean irradiance can be unrelated to area.

L'irradiation moyenne de la couche superficielle a été calculée en pourcentage d'irradiation incidente à l'aide des profondeurs de Secchi et des profondeurs de la thermocline dans deux séries de lacs : (1) 20 lacs presque circulaires du nord-ouest du Minnesota aux États-Unis, échantillonnés au cours d'une saison similaire, et (2) mille cent quarante lacs de formes hétérogènes en Ontario au Canada, échantillonnés pendant la saison libre de glace. Dans les deux cas, l'épaisseur de la couche superficielle augmentait proportionnellement à la superficie du lac. En conséquence, les lacs les plus étendus affichaient une irradiation moyenne inférieure dans leur couche superficielle. La profondeur du lac avait moins d'influence, mais en Ontario l'atténuation diminuait et l'irradiation moyenne augmentait avec l'augmentation de la profondeur. La superficie du lac (exprimée en logarithme) expliquait environ la moitié de la variation de l'irradiation moyenne des échantillons homogènes des lacs du Minnesota, tandis que la superficie et la profondeur expliquaient moins, à eux deux, la variation dans les échantillons hétérogènes des lacs ontariens. La forme, c'est-à-dire le développement des rivages, s'est révélée sans importance comparativement aux mesures grossières de la taille : superficie et profondeur. L'irradiation moyenne est reliée de façon complexe à la taille du lac : lorsque la profondeur augmente lentement avec la superficie, les gros lacs peuvent avoir une moindre irradiation moyenne en surface. Mais lorsque la profondeur augmente rapidement avec la surface, l'irradiation moyenne peut ne pas être reliée à la superficie.

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One of the oldest limnological principles is that basin morphometry influences lake metabolism (Huitfeld-Kaas 1898; Thienemann 1925; Naumann 1932). Morphometry influences the loading, dilution, and recycling of nutrients, the rate of mixing, and the flux of heat in and out of a lake. Relationships between morphometry and nutrient metabolism have been most thoroughly explored. Cornerstones of this work are (1) the ratio of total catchment area to lake volume correlates with nutrient concentration and thus also with production (Schindler 1971a) and (2) the ratio of the area of the epilimnion in contact with the sediments to the epilimnion volume influences nutrient resupply and thus also production (Fee 1979, 1980; Patalas 1980).

It is occasionally acknowledged that morphometry influences a lake's light climate as well, but morphometry-light relationships are poorly defined. Sakamoto (1966) suggested that light is more limiting in shallow, unstratifying, eutrophic lakes than in deep, stratifying, oligotrophic lakes because of differences in transparency. However, he also suggested that during holomixis photosynthesis was more light-limited in deeper lakes because of their greater depths of mixing. He did not compare stratified lakes of differing size. There has been little study since Sakamoto, and any relationship between basin morphometry and light climate remains unproven.

The underwater light field is complex, a reality which can frustrate attempts to identify fundamental relationships. Nevertheless, a single summary parameter is the mean of the photosynthetically active radiation (PAR) in the surface layer (Riley 1957), $\bar{E}$. $\bar{E}$ is a function of transparency and thermocline depth (see equation (1) below). Larger, deeper lakes may have relatively high $\bar{E}$ if these are more transparent than smaller, shallower lakes. However, mixed layer depth increases with lake area (Krambeck 1974; Gorham 1980; Cruickshank 1984; Gorham and Boyce 1989), and this would lower mean irradiance in larger lakes. Higher transparency coincides with deeper mixed layers (Kling 1988). Thus, it is not known whether larger lakes have higher or lower $\bar{E}$ than smaller lakes.

In this paper I examine how mean irradiance in the surface layer varies with lake morphometry. Specifically, I ask the question: Is the light climate generally brighter or dimmer as lakes go from small to large? And additionally: Which specific morphometric parameters drive the light–morphometry relationship?

TABLE 1. Summaries of individual parameters. See text for explanation of symbols.

	Sample size	Mean	Median	Minimum	Maximum
Minnesota					
A_0 (ha)	20	399.4	30.0	1.8	2085.0
Z_{max} (m)	20	22.0	17.0	6.4	64.9
T (m)	20	6.1	4.6	2.3	16.5
Z_{sd} (m)	20	3.2	3.2	1.2	6.5
Ontario					
A_0 (ha)	1140	478.6	58.4	1.3	34807.9
P (km)	1140	16.9	5.3	0.5	511.0
Z_{max} (m)	1140	22.0	18.0	0.3	213.5
$\bar{z}$ (m)	1140	7.3	5.9	0.7	41.2
Z_{sd} (m)	1140	4.4	4.1	0.5	15.3
T_1 (m)	914	6.0	5.4	0.8	17.3
T_2 (m)	824	5.6	5.4	0.8	15.6
SLD	1140	2.4	2.0	0.9	18.1
Depth ratio	1140	0.35	0.34	0.04	0.91

Methods

Unpublished data for 20 lakes in northwest Minnesota, USA were supplied by Dr. Eville Gorham (Department of Ecology and Behavioral Biology, University of Minnesota, Minneapolis, MN 55455). The relationship between morphometry and thermocline depth in these lakes has been described (Gorham and Boyce 1989). These lakes all had similar shape (circular or subcircular) and were sampled during maximum heat content (= last week of July to third week of August). Thermocline depth was taken as the position of the maximum temperature gradient.

Information for over 10 000 lakes in Ontario, Canada have been obtained by the Ontario Aquatic Habitat Inventory Program (Goodchild and Gale 1981) (G. Gale, Fish Community and Habitat Section, Fisheries Branch, Whitney Block, Queen's Park, Toronto, Ont. M7A 1W3, unpubl. data). From these, a subset of 1140 was taken by selecting sites fulfilling all of the following: (1) water colorless (judged visually) for at least one station; (2) percent cloud cover ≤80 during recording of Secchi depth; (3) morphoedaphic index (total dissolved solids/mean depth; Ryder et al. 1974) <40; (4) pH ≥5.0; (5) total fixed endpoint alkalinity >6.8 mg/L; and (6) specific conductance <30 μS/cm. These criteria tended to exclude lakes which were acidified, bog-influenced, saline, or eutrophied. In the lakes selected, shape varied widely, and sampling had been performed throughout the ice-free season.

Data for the Minnesota lakes consisted of (Table 1): area (A_0), maximum depth (z_{max}), thermocline depth (T) (to the nearest 0.1 m), and Secchi depth (z_{sd}). Data for the Ontario lakes were: A_0, perimeter (P), z_{max}, mean depth ($\bar{z}$), and a set of one to 10 measurements of z_{sd} along with temperature profiles. Temperature was recorded at intervals typically spaced at 1.5 m. To obtain thermocline depths, temperature profiles were scanned from the surface downward to locate (1) the midpoint between the two depths with maximum temperature gradient (T_1) and (2) the shallowest midpoint between two depths with a gradient of at least 1.5°C/m (T_2). Sites with T_1<1.5°C/m or with <2 depth readings above or below T_1 or T_2 were excluded.

Shape was computed as departure from circularity, or shoreline development (SLD) (Wetzel 1983):

TABLE 2. Parameters selected by stepwise variable selection and multiple regression. To be included, variables had to have $F > 4$ ($p<0.001$ for all). Correlations (r; partial correlations where the number of variables > 1) were calculated for all selected variables ($p > 0.001$ for all except for $\bar{E}_2\%$, $log(z_{max})$ and $log(\bar{z})$ where $p = 0.02$.

Parameter	Coefficient (SE)	Correlation (r)
(K) Minnesota		
constant	.53(.06)	—
(K) Ontario		
constant	.75(.03)	
$log(A_0)$	.09(.01)	.25
$\log(z_{max})$	−.22(.04)	−.15
$log(\bar{z})$	−.23(.04)	−.16
SLD	−.02(.006)	−.08
(T) Minnesota		
$log(A_0)$	3.57(.38)	.91
(T_1) Ontario		
constant	2.93(.19)	—
$log(A_0)$	1.03(.07)	.42
$log(\bar{z})$	1.45(.23)	.20
(T_2) Ontario		
constant	2.67(.20)	—
$log(A_0)$	1.07(.08)	.44
$log(\bar{z})$	1.03(.24)	.15
($\bar{E}$) Minnesota		
constant	.56(.05)	—
$log(A_0)$	−.11(.02)	−.73
($\bar{E}_1 1\%$) Ontario		
constant	.46(.01)	—
$log(A_0)$	−.07(.005)	−.41
$log\ (\bar{z})$	.12(.02)	.24
($\bar{E}_2\%$) Ontario		
constant	.45(.02)	—
$log(A_0)$	−.08(.006)	−.43
$log(z_{max})$	.08(.03)	.08
$log(\bar{z})$	.07(.03)	.08

$$\text{SLD} = \frac{P}{2A_0\pi}$$

Depth ratios ($\bar{z}/z_{max}$, Table 2) indicated that most Ontario lakes had hyperbolic or sinusoidal basin shape (defined in Carpenter 1983) while less than 8% had ellipsoid-shaped basins.

Calculation of Mean Irradiance

PAR in a surface layer decreases approximately exponentially with depth; departures from perfect exponential decay are from spectral modification and vertical heterogeneity in attenuation and scattering. Mean irradiance is a function of: (1) incident irradiance, (2) transmission through the air/water interface, (3) attenuation, and (4) the thickness of the surface layer. Factors 1 and 2 are extrinsic to the water column; factors 3 and 4 are intrinsic. Extrinsic features (such as meteorology), though of obvious importance to light climate, will not be considered here. Intrinsic factors can be isolated by expressing mean PAR as a percent of irradiance at depth zero.

Scalar irradiance (E) is the integral of the radial distribution of PAR around a point over all directions about the point (terminology follows Kirk 1983). The rate of attenuation of E with depth is K, a broadband vertical attenuation coefficient. The mean scalar irradiance ($\bar{E}$) in a water column with lower boundary at depth $z = T$ is found by taking the first moment (Riley 1957):

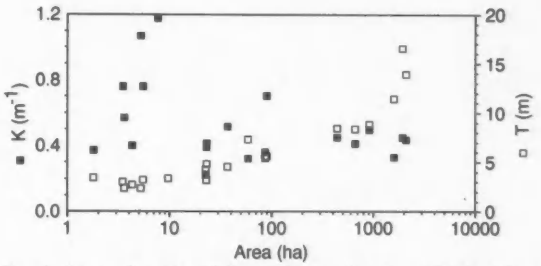

FIG. 1. Attenuation (K) and depth of the surface layer (T) plotted versus surface area (A_0) for 20 Minnesota lakes.

$$(1)\quad \bar{E} = \frac{E_0}{T}\int_0^T e^{-Kz}\,dz = \frac{E_0\,(1-e^{-K\cdot T})}{K\cdot T}$$

where E_0 equals the scalar irradiance just below the surface. The dimensionless product $K\cdot T$ has been called the "optical depth" (Talling 1957, 1971). Mean irradiance can be expressed as a percent of the irradiance at depth zero;

$$(2)\quad \bar{E}\% = 100\,(\bar{E}/E_0) = \frac{100\,(1-e^{-K\cdot T})}{K\cdot T}\ .$$

$\bar{E}\%$ is determined solely by two intrinsic parameters: K and T.

An approximate value for K can be obtained from the Secchi depth (Poole and Atkins 1929; Graham 1966; Schindler 1971b; Kirk 1983; Reynolds 1984; Rott 1986):

Where ϕ is an empirical constant. This relationship is known to be inexact. Physical derivation shows that ϕ/z_{sd} is proportional to the sum of K and the beam attenuation coefficient, c (Duntley and Preisendorfer 1952; Tyler 1968; Preisendorfer 1986). But, since K and c tend to covary (Kirk 1983), the correlation between K and z_{sd} can be good (Holmes 1980; Rott 1986; Bukata et al. 1988). Dr. Robert O. Megard (Department of Ecology and Behavioral Biology, University of Minnesota, Minneapolis, MN 55455) has determined a value of 1.54 for ϕ from measurements of Secchi depths and attenuation of the most penetrating waveband. This value is similar to the 1.44 of Holmes (1970) recommended by Kirk (1983). Here, I have calculated $\bar{E}\%$ from equations (1) and (2) using 1.54 for ϕ in equation (3).

If, (1) the occlusion of incident light by obstructions around the lake's shoreline and (2) the extent of the area where the lake bottom is shallower than the depth of the surface layer are both small, equation (2) gives $\bar{E}\%$ for the lake as a whole. For the Minnesota lakes, equations (2) and (3) were used to obtain $\bar{E}\%$. For the Ontario lakes, T_1 and T_2 each yielded an estimate of mean irradiance, $\bar{E}_1\%$ and $\bar{E}_2\%$.

Data Analysis

A_0, z_{max}, and $\bar{z}$ were $\log_{10}$ transformed to stabilize variances, to improve linearity, and to reduce the leverage of the few very large lakes. These three variables and SLD (untransformed) were available as potential morphometric predictor variables for Ontario while only A_0, and z_{max} were available for Minnesota. Simple regressions identified statistically significant ($p<0.05$) relationships (slopes) between individual pairs of variables. Multiple regressions using a stepwise variable selection procedure (Weisberg 1985) identified which of the morphometric predictors that were related to K, T, or $\bar{E}\%$ when the potential influence of all possible predictors were taken into account simultaneously.

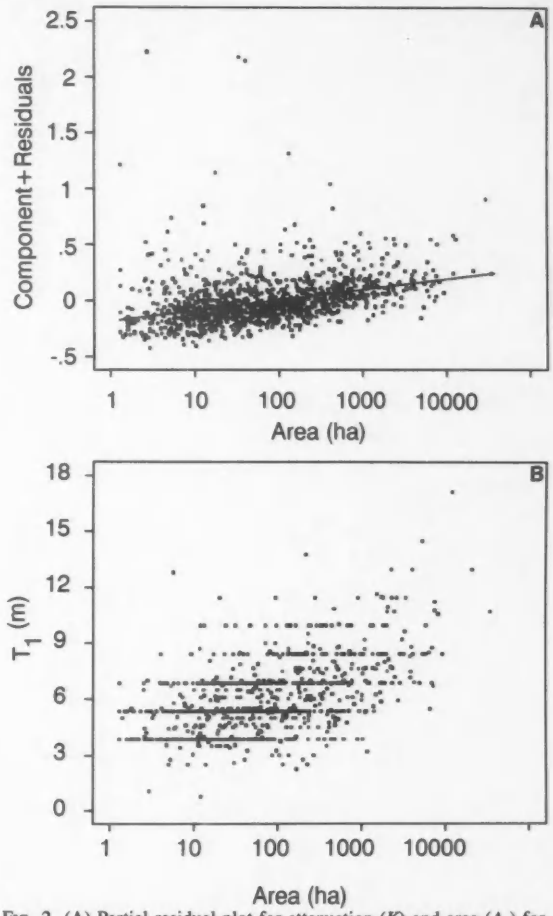

FIG. 2. (A) Partial residual plot for attenuation (K) and area (A_0) for 1140 Ontario lakes. The slope of the line is the regression coefficient (Table 2). The separate points indicate the residuals around that line while adjusting for all the other predictors (Weisberg 1985). (B) Depth of the surface layer (T_1) and area (A_0) for 914 Ontario lakes. The plot of T_2 and A_0 was similar.

Results

Morphometry and Attenuation

In Minnesota lakes smaller than 100 ha in surface area, K was highly variable from lake to lake, but was uniformly low for larger lakes (Fig. 1). Significant linear trends of K versus either surface area or depth could not be found: K was not significantly related to either $log(A_0)$ or to $log(z_{max})$ singly (simple regressions, $p>0.1$ for both) or in combination (Table 2).

In contrast, significant trends were seen in the Ontario lakes. K was related to z_{max} and $\bar{z}$ singly ($p<0.001$ for both), though not to area or SLD. All four were significant when in

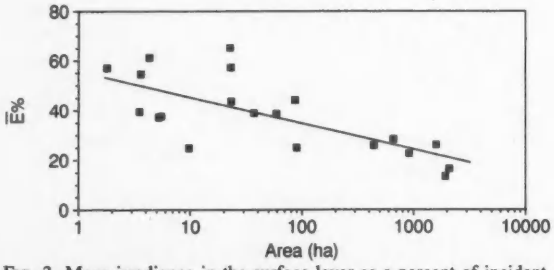

FIG. 3. Mean irradiance in the surface layer as a percent of incident ($\bar{E}$%) and area (A_0) for 20 Minnesota lakes. Line comes from coefficients in Table 2.

combination (Table 2). Increasing lake "size" had conflicting effects on attenuation: increasing area increased K, while increasing depth decreased K (Table 2). Increasing SLD also decreased K. Thus, for lakes of similar depth and shape, increased area led to higher attenuation, while for similar area and shape, increasing depth led to lower attentuation. $Log(A_0)$ was not significant in simple pairwise regression, but in multiple regression, when all four parameters were accounted for, $log(A_0)$ was the morphometric parameter most closely related to K, followed in order by $log(z_{max})$, $log(\bar{z})$, and SLD (Table 2). Apparently, area and depth counteracted in the simple regressions, but in multiple regression their separate influences were more clearly seen. The effect of $log(A_0)$ after factoring out the influence of the other variables can be visualized in a "partial residual plot" (Fig. 2A), which shows that in the majority of lakes, K is well-predicted by this combination (Table 2) of morphometric parameters. The few outlying lakes are primarily small in area and have much greater attenuation than predicted based on morphometry alone. Nevertheless, much of the variation in K was left unexplained by morphometry. The lack of significance between K and morphometry in the Minnesota lakes may well have reflected low statistical power rather than lack of an underlying trend.

Morphometry and Depth of the Surface Layer

In both sets of lakes, morphometry was more closely related to the depth of the surface layer than it was to attenuation. In the Minnesota lakes, T increased with increasing area ($p<0.001$) (Fig. 1) and depth ($p<0.001$) singly. However, area, but not depth, was selected (Table 2), indicating that once the effect of area was accounted for, depth did not explain a significant additional amount of variation. $Log(A_0)$ alone explained 83% of the variation in T (r^2, Table 2), even without taking into account a pronounced nonlinearity in the largest lakes (Fig. 1).

In simple regressions of Ontario Lakes, T_1 and T_2 both increased with increasing area, increasing maximum depth, and increasing mean depth ($p<0.001$ for all six combinations). Area and mean depth were selected for both (Table 2). Basin shape was unimportant in influencing surface layer depth. Depth of the surface layer was made greater by either increasing A_0 or increasing $\bar{z}$, with more of the variation explained by area than by depth. The total amount of variation in depth of the surface layer that was explained by morphometry was lower in the Ontario lakes than in the Minnesota lakes (Table 2; Fig. 2B).

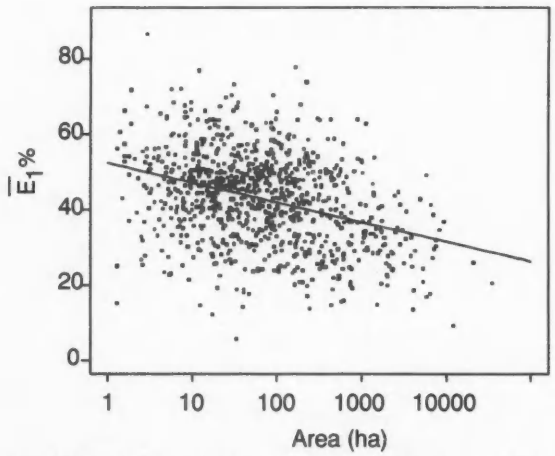

FIG. 4. Mean irradiance in the surface layer ($\bar{E}_1$%) and area (A_0) for 914 Ontario lakes.

Morphometry and Mean Irradiance in the Surface Layer

In the Minnesota lakes, $\bar{E}$% was related to both area ($p<0.001$) and depth ($p=0.001$) singly while only area was chosen by multiple regression (Table 2). Lakes larger in area had lower $\bar{E}$% (Fig. 3). For small lakes, mean irradiance in the surface layer was more variable than for larger lakes (Fig. 3). Approximately half (53%) of the variation in $\bar{E}$% was explained by area alone (r^2, Table 2).

Patterns of lake morphometry and mean irradiance in the Ontario lakes were remarkably similar to the Minnesota lakes. Singly, both $\bar{E}_1$% and $\bar{E}_2$% were related to area ($p<0.001$) but not to depth. In multiple regressions, lake area was again the most closely related morphometric parameter (Table 2). Increasing area again led to low mean irradiance (Table 2; Fig. 4). Variance in mean irradiance was again greater for smaller lakes than for larger ones (Fig. 4). Slopes of regression lines of mean irradiance on area were similar for Minnesota and Ontario (Table 2). Perhaps because of the greater sample size, lake depth was chosen for the Ontario lakes while not for the Minnesota lakes. Increasing lake depth ($\bar{z}$ for $\bar{E}_1$% or $\bar{z}$ plus z_{max} for $\bar{E}_2$%) led to higher mean irradiance (Table 2).

In the Ontario lakes, increasing area led to lower mean irradiance for two reasons: greater attenuation and deeper surface layers. Increasing depth simultaneously lowered attenuation and increased the depth of the surface layer. Apparently, the former overwhelmed the latter, as increasing depth on balance increased, not decreased, mean irradiance. Basin shape was unimportant in either data set.

Discussion

In this study, stratified lakes of larger area had lower mean irradiance in the surface layer (Figs. 3, 4). This was primarily because of increasing thickness of the surface layer. In addition, increased attenuation with increasing area augmented the effect of thermocline depth in the Ontario lakes, but not in the Minnesota lakes.

Lake depth also influenced mean irradiance, but in a more complex manner. In the Minnesota lakes, effects of depth on surface layer depth and mean irradiance became insignificant

after area was accounted for. In the Ontario lakes, both mean and maximum lake depth were significantly related to mean irradiance, even after the effect of area was included. Lake depth was related to both thermocline depth and to attenuation. As with increasing area, increasing depth made the surface layer deeper. Thus, considering only the role of morphometry in controlling thermocline depth, both increasing area and increasing depth should lower mean irradiance, meaning that "larger" lakes would have lower mean irradiance than "smaller" lakes. However, the second influence of increasing depth was to reduce attenuation. Increasing depth therefore had two opposite influences on mean irradiance. Lowered attenuation was more influential than increased surface layer depth; on balance, mean irradiance was positively related to depth.

Lake "size" thus has a complex control of light climate, with area and depth exerting opposite influences. It follows that a negative trend of mean irradiance with increasing area depends upon the relationship between depth and area. In particular, if depth increased quickly with area, increasing surface layer depth could be offset by lowered attenuation, leaving no relationship between lake area and mean irradiance. Such seems to be the case in the following example.

Fee (1979) examined morphometry and primary productivity for 18 lakes (16 with data for thermocline depth) in the Experimental Lakes Area, Canada. In contrast to the two sets of lakes in the present study, mean irradiance ($\bar{E}\%$) and area ($log_{10}(A_0)$) were unrelated in the ELA (experimental lakes area) lakes ($r^2 = 0.008$; $n = 16$, $p = 0.27$). Regressions of depth on area (significant for all three sets of lakes) suggest that area and depth interact in different ways in the ELA lakes than they did in the Minnesota and Ontario lakes of the present study:

Ontario:	$log(\bar{z}) = .55 + .12\ log(A_0)$	$r = .35$
	$log(z_{max}) = .95 + .16\ log(A_0)$	$r = .44$
Minnesota:	$log(z_{max}) = .89 + .21\ log(A_0)$	$r = .78$
ELA:	$log(\bar{z}) = .27 + .42\ log(A_0)$	$r = .74$
	$log(z_{maz}) = .59 + .46\ log(A_0)$	$r = .76$

The slopes in the ELA lakes (.42–.46) are considerably greater than those for the Ontario (.12–.16) and Minnesota (.21) lakes, which may explain why $\bar{E}\%$ was not related to area in the ELA lakes.

Morphometric parameters had contrasting success in predicting mean irradiance in the Minnesota and Ontario lakes. In the Minnesota lakes, $log(A_0)$ alone explained more than 50% of the variation in $\bar{E}\%$. In the Ontario lakes, however, area and depth together explained considerably less of the variation. Some of this difference is attributable to the precisions of measurement of thermocline depth (Minnesota: ± 0.10 m; Ontario generally ± 1.5 m: note horizontal banding in Fig. 4). But also, the Ontario lakes were more widely scattered geographically and in terms of sample time than the Minnesota lakes.

Conclusions

The importance of the depth of the thermocline in controlling the light climate of a water column has long been recognized in freshwater and marine settings (Sverdrup 1953; Riley 1957; Talling 1971). The suggestion is made here that lake morphometry, especially surface area, influences the mean underwater irradiance in the surface layer mainly because morphometry influences thermocline depth. Stratifying lakes large in area tend to have low mean irradiance in their surface layers. Smaller lakes are more variable, but frequently have relatively bright mixed layers. However, basin geometry must be taken into account; when depth increases rapidly with area, an opposing influence of depth, lowering attenuation, may eliminate the relationship between area and mean light.

Brylinski and Mann (1973) examined IBP data from 55 lakes and reservoirs distributed worldwide and asserted that "variables related to solar energy input have a greater influence on production than variables related to nutrient concentration." This conclusion must certainly have arisen, in part, because areal, not volumetric, production was studied. Later comparative studies (Schindler 1971a; Fee 1979) have concluded that nutrient dynamics are closely coupled to volumetric production. It is not the purpose of this paper to echo the assertion of Brylinski and Mann (1973); instead, light–morphometry relationships can be blended into nutrient–morphometry ones to produce a more complete picture of the role of morphometry in controlling lake metabolism.

Both Schindler (1971a) and Fee (1979) found some ratio of area to volume which correlated well with production. Because of simple scaling laws, small lakes, often having high $\bar{E}\%$, would have higher area:volume ratios than larger lakes, which often have lower $\bar{E}\%$. This elementary argument suggests that irradiance reinforces nutrient dynamics, and suggests that small basins are more productive than large ones for both energy-related and nutrient-related reasons.

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Serial Passage of Larval *Pseudoterranova decipiens* (Nematoda:Ascaridoidea) in Fish

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In one experiment, 24 brook trout (*Salvelinus fontinalis*) in fresh water at 11 ± 1°C were each orally infected by intubation with two third-stage larvae of "sealworm" (*Pseudoterranova decipiens*) harvested from the flesh of sea raven (*Hemitripterus americanus*) and small Atlantic cod (*Gadus morhua*). In a second experiment, 27 cod in sea water at 0°C were each force fed, under anaesthesia, four *P. decipiens* larvae held in a capelin "purse"; these larvae were harvested from large, commercial size cod. Sequential reinvasion by the same *P. decipiens* larvae was achieved in both of the serial passage experiments. In brook trout, larvae sequentially reinvaded a maximum of two fish, with larvae of cod origin being the more successful at first passage (62.5%) than those of sea raven origin (31.3 and 37.5%). In cod, larvae also achieved sequential reinvasion of a maximum of two fish; the relatively lower success rates of 22.2% (first passage) and 9.1% (second passage) probably reflect the low temperature (0°C) at which the experiment was conducted.

Lors d'une expérience, 24 ombles de fontaine (*Salvelinus fontinalis*) en eau douce à 11 ± 1°C ont été infectées par intubation orale avec deux larves de ver de phoque au troisième stade (*Pseudoterranova decipiens*) prélevées dans la peau d'hémitriptères d'Amérique (*Hemitripterus americanus*) et de morues de l'Atlantique de petite taille (*Gadus morhua*). Lors d'une deuxième expérience, 27 morues placées dans l'eau de mer à 0°C ont chacune ingéré de force, sous anesthésie, quatre larves de *P. decipiens* dans des « poches » faites de capelans; ces larves avaient été prélevées sur de grandes morues de taille commerciale. La réinvasion séquentielle par les mêmes larves de *P. decipiens* a été obtenue dans les deux expériences de passage séquentiel. Chez l'omble de fontaine, les larves ont séquentiellement envahi un maximum de deux sujets, celles provenant des morues ayant plus de succès au premier passage (62,5 %) que celles provenant des hémitriptères (31,3 % et 37,5 %). Chez la morue, il y a aussi eu une réinvasion séquentielle d'un maximum de deux poissons; le taux de succès relativement inférieur de 22,2 % (1er passage) et de 9,1 % (2e passage) est probablement le résultat de la température inférieure de l'eau (0°C) dans les expériences sur la morue.

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It is known that third-stage larvae (L_3) of "sealworm" *Pseudoterranova decipiens* in the flesh of one fish host can be transmitted (through predation) to the flesh of a second fish host (Scott 1954). What is not known, however, is how many times this fish-to-fish transfer can occur. Larvae in the flesh of the sea raven (*Hemitripterus americanus*) appear in time to become heavily encapsulated with host material, and ultimately to die with the development of black, necrotic material (unpubl. data). Small Atlantic cod (*Gadus morhua*), which feed mainly on crustaceans, harbour relatively few L_3 but these (presumably in a fish host for the first time) are found throughout the musculature, indicating an ability to penetrate deep into the flesh. As the cod grow they become largely piscivorous and presumably acquire L_3 that were previously in the prey fish, as the number of larvae increases with fish size. Reports of the intensity of L_3 in larger cod, however, indicate that there is a decrease in the density, i.e. number of worms/unit weight; moreover, the number of worms in the belly flaps of the fish increases. These observations are consistent with the hypothesis that there is a limit to the number of times that larvae can be transmitted from fish to fish. Two experiments were designed to test this hypothesis. Due to the various difficulties involved with experimental infections and holding infected fish, sampled sizes are small.

Materials and Methods

Sources of *P. decipiens* L_3

For experiments 1, sea raven (*Hemitripterus americanus*) and Atlantic cod (*Gadus morhua*) caught in Passamaquoddy Bay, New Brunswick, were kept alive in fish tanks, with running sea water, until killed and dissected for the recovery of *Pseudoterranova decipiens* L_3. Larvae from 3.0 to 4.1 (mean 3.6) cm long were carefully freed from any host capsule material.

For experiment 2, *P. decipiens* L_3, 3.0 to 4.6 (mean 3.9) cm long, were removed from Atlantic cod fillets at a commercial

fish processing plant in Newfoundland, and dissected free from any host capsules.

All larvae used in the two experiments were morphologically identified as *P. decipiens* and judged to be viable on the basis of movement and absence of obvious physical damage. Larvae were stored in 0.85% NaCl solution at 4°C for up to 24 h prior to use.

Recipient Fish

For experiment 1, *Salvelinus fontinalis*, obtained from a local hatchery and 18-mo-old at the start of the experiment (average length, 27.0 cm), were kept in fresh water at 11 ± 1°C. The brook trout had been fed only pelleted food and were free, therefore, from *P. decipiens*.

For experiment 2, 50 Atlantic cod (25 males, 25 females) were obtained from a commercial farm on the east coast of Newfoundland; they ranged in length from 34.5 to 64.0 cm. As a control, 16 fish were killed and examined for *P. decipiens* by dissection and pepsin-HCl digestion (see Smith and Wootten 1975); none was infected. The remaining 34 cod were used for the serial transfer experiment, during which the sea water temperature was 0°C.

Infection Procedure

At the beginning of each experiment, two larvae were introduced via stomach tube into each of eight fish (experiment 1) or four larvae in a capelin "purse" were force fed to 27 cod under MS222 anaesthesia (experiment 2). The fish were left for 7 d (experiment 1) or for 7 to 29 d (experiment 2) after infection then killed and examined for larvae. Any larvae recovered were stored in 0.85% saline for a period up to 2 d, and only live, apparently undamaged larvae were used to reinfect new fish. The process was repeated until either there were no larvae or the larvae recovered were dead.

Experiment 1 was done three times: once with larvae from *G. morhua* (experiment 1a), and twice with larvae from *H. americanus* (experiments 1b and 1c).

Recipient fish were killed by anaesthetic overdose, and carefully examined for nematodes in the stomach and intestinal lumen and wall tissues, visceral organs, and musculature. In experiment 2, manual dissection was supplemented by pepsin-HCl digestion of viscera and flesh. The location of larvae and condition of surrounding tissues was recorded.

Results

Experiment 1a

The eight trout were examined 7 d after infection with larvae from *G. morhua*, (first passage), and 10 larvae (62.5%) were recovered from the musculature (Table 1). Of these 10 larvae, eight were used to infect four additional trout (second passage, two larvae each). After 7 d, two larvae (25.0%) were recovered from the four fish. A subsequent experimental infection of another trout with the two *P. decipiens* larvae (third passage) was not successful (Table 1).

Experiment 1b

The eight trout examined 7 d after infection with larvae from *H. americanus* (first passage) yielded five larvae (31.3%) (Table 1). These five larvae were used to infect three additional trout (second passage). After 7 d, only one larva (20.0%) was recovered. A subsequent experimental infection of another trout with this larva (third passage) was not successful (Table 1).

Experiment 1c

Seven days after infection of eight trout with larvae from *H. americanus* (first passage), six larvae (37.5%) were recovered (Table 1). These six larvae were used to infect three additional trout (second passage); no larvae were recovered 7 d later (Table 1).

Experiment 2

Of the 108 larvae introduced into the stomach of 27 cod (first passage, four per fish), 71 (65.7%) were accounted for at examination from 7 to 29 d later. Forty-seven larvae (43.5%) had remained in the gastro-intestinal tract lumen: 46 in the stomach lumen, and one in the intestinal lumen; despite the long sojourn, 18 (38.3%) of these 47 larvae were still alive and apparently undamaged. Twenty-four (22.2%) of the original 108 larvae had successfully penetrated host tissues (Table 2), and two of these were damaged during recovery. Six of the 24 larvae had penetrated the stomach wall and were found encapsulated in association with the stomach serosa; two encapsulated larvae were found elsewhere in the body cavity, one associated with the liver, the other with the pyloric caeca; the remaining 16 larvae were found in the flesh, none of them encapsulated. Of the 22 undamaged larvae used for second passage in six cod (three or four per fish), 12 (54.5%) were accounted for after 14

TABLE 1. Serial passage of *Pseudoterranova decipiens* L^3 in *Salvelinus fontinalis* at 11 + 1°C.

Experiment	Source of larvae	Passage	Number of recipient fish	Total number larvae introduced	Number (%) larvae passaged successfully
1a	*Gadus morhua*	1st	8	16[a]	10(62.5)
		2nd	4	8[a]	2(25.0)
		3rd	1	2	0(0.0)
1b	*Hemitripterus americanus*	1st	8	16[a]	5(31.3)
		2nd	3	5[b]	1(20.0)
		3rd	1	1	0(0.0)
1c	*Hemitripterus americanus*	1st	8	16[a]	6(37.5)
		2nd	3	6[a]	0(0.0)

[a] two larvae/fish.
[b] One, two, and two larvae/fish.

TABLE 2. Experiment 2: serial passage of *Pseudoterranova decipiens* L_3 in *Gadus morhua* at 0°C.

Source of larvae	Passage	Number of recipient fish	Total number larvae introduced	Number (%) larvae passaged successfully
Gadus morhua	1st	27	108[a]	24(22.2)
	2nd	6	22[b]	2(9.1)
	3rd	1	2	0(0.0)

[a]Four larvae/fish
[b]Three or four larvae/fish.

d. Ten larvae (45.5%) were found dead in the stomach lumen. The other two (9.1%) had successfully penetrated host tissues; one was found encapsulated in association with the pyloric caeca, the other occurred, nonencapsulated, in the flesh (Table 2). Neither of these two larvae was recovered from a cod (third passage) after 12 d (Table 2).

Discussion

There appears to be no earlier record in the literature of serial passage through experimental fish using *P. decipiens* larvae. However, Wootten and Smith (1975) tried serial infections with larvae of a related ascaridoid, *Anisakis simplex*. These larvae, removed from herring (*Clupea harengus*), were introduced into brown trout (*Salmo trutta*) and rainbow trout (*Oncorhynchus mykiss*) in fresh water. In both cases, the larvae failed to pass through experimental fish more than twice. The infection procedure was similar to that used in the present study.

Penetration of the stomach wall, movement through the body cavity, and penetration of the musculature of a fish presumably requires energy and, possibly, the ability to produce histolytic enzymes. It is reasonable to assume that such activity can only occur a limited number of times, unless the larvae are able to feed and replenish their energy and enzyme levels.

As *P. decipiens* larvae appear to feed and grow in fish musculature, on theoretical grounds they may be able to replenish their energy stores and be capable of passing from fish to fish several times. However, an infinite number of passages appears to be unlikely; the increasing number of *P. decipiens* larvae found in the body cavity and body flaps of older cod suggests that these larvae have insufficient energy stores or, possibly, enzymes to reach the musculature, having passed through previous fish hosts on perhaps two of three occasions.

Although larvae removed from both cod and sea raven were not able to reinvade sequentially more than two brook trout in experiment 1, at first passage almost twice as many larvae of cod origin were recovered (62.5%) compared with those of sea raven origin (31.3 and 37.5%). The larvae from cod in experiment 1 came from small, young fish which had probably acquired the infection directly by eating an invertebrate host. Larvae from sea raven, on the other hand, probably had naturally passed through at least one fish host prior to infecting this host, as sea raven are piscivorous even when quite small. In experiment 2, the *P. decipiens* larvae were harvested from large, relatively old, commerical size fish which, like the sea raven, are likely to have acquired the larvae by feeding largely on fish. Another major reason for the low success rate in experiment 2, in terms of both the proportion penetrating at first and subsequent passages, and the number of sequential passages achieved, is likely to be the low ambient sea water temperature, the experiment having been conducted in the period January–March. In both experiments, the numbers of recipient fish and larvae available were low, especially at the third passage, and are of doubtful statistical significance, therefore. It would be of interest to repeat the experiment with Atlantic cod as donors and recipients of *P. decipiens* L_3 but at a higher temperature than 0°C.

Other factors which might have affected the success of serial passage are damage to larvae in the experimental infection process (both experiments), and in experiment 1, larvae harvested from sea raven may have suffered a more severe host response than those harvested from cod.

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Size Selection of Snappers (*Lutjanidae*) by Hook and Line Gear

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The most commonly used theoretical models of gear selection have been the logistic and normal curves. These are usually applied to trawls and gillnets, respectively. In contrast, little critical work has been completed concerning the selective properties of fish hooks, although both types of selectivity curves have arbitrarily been applied to hook catch data in the literature. No study has clearly demonstrated the actual form of a selection curve for hooks. To determine which type of curve (logistic or normal) best describes the selective sampling characteristics of fish hooks, an experiment was conducted in the Marianas Islands during 1982–84. During all fishing operations two different sizes (#20 and #28) of circle fish hooks were fished simultaneously and in equal number. Under these conditions, the length specific ratios of snapper (*Lutjanidae*) catches taken by the two hook sizes provided a basis for distinguishing which model was most appropriate. Results showed that neither model in its simplest form depicted hook selectivity well. While small hooks caught substantially more small fish, large hooks were somewhat more effective in capturing the larger size classes.

Les modèles théoriques de la sélectivité des engins les plus couramment utilisés sont les courbes logistiques et normales, qui sont généralement appliquées respectivement aux chaluts et aux filets maillants. On a par contre effectué très peu d'études critiques sur les propriétés sélectives des hameçons, bien que les deux types de courbes de sélectivité aient été arbitrairement appliquées dans certaines travaux aux données concernant les prises faites à l'hameçon. Aucune étude n'a clairement démontré la forme réelle d'une courbe de sélectivité des hameçons. Pour déterminer quel type de courbe (logistique ou normale) décrit le mieux les caractéristiques de prélèvement des hameçons, nous avons mené entre 1982 et 1984 une expérience aux îles Mariannes. Pendant toutes les opérations de pêche, on a utilisé des hameçons circulaires de deux tailles différentes (n° 20 et n° 28) simultanément et en nombre égal. Dans ces conditions, le rapport de longueur des captures de vivaneaux (Lutjanidés) effectuées avec les deux hameçons a servi de base pour déterminer quel modèle convenait le mieux. Les résultats ont montré qu'aucun des modèles sous sa forme la plus simiple ne décrivait correctement la sélectivité des hameçons. Tandis que les petits hameçons capturaient nettement plus de poisson de petite taille, les gros hameçons étaient légèrement plus efficaces pour la capture des classes de taille supérieure.

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Many of the world's most important fisheries use hook-and-line gear. Examples include the Japanese, Korean, and Taiwanese distant-water longline fisheries for tuna (Brock 1962), the west coast salmon troll fishery, and the groundline fishery for Pacific halibut (Myhre and Quinn 1984). Likewise, all recreational pole-and-line fisheries, both marine and freshwater, are based on angling with fish hooks. However, despite this widespread use, the sampling characteristics of hook-and-line gear are not well understood. There has been no coherent development of theory concerning the manner in which fish hooks sample a population (Clark 1960; Pope et al. 1975). As a result, it is difficult, if not impossible, to deduce the age or size structure of a population from a single hook-and-line catch sample. One reason for this is that gear selection models developed for trawl and gillnet fisheries have been applied to hook-and-line based fisheries with little or no verification that these models are, under a broad range of conditions, well-suited to the task (e.g. Koike and Kanda 1978; Munro 1983).

One of the earliest works on hook selection showed that length–frequency samples of cod caught with longline gear were shifted to smaller sizes than similar samples that were taken with purse seine gear. The latter gear type was believed to representatively sample the population's size structure (Rollefsen 1953), suggesting that the selectivity curve for hooks must ultimately decrease with increasing size of fish. Such a pattern is in some ways similar to selection curves for gillnets, which show rising and falling limbs. Gillnet selectivity has usually been described with the symmetrical normal curve (Hamley 1975). Other studies have shown similar results from hook-and-line fishing (haddock in McCracken 1963; Koike et al. 1968; Goose Islands halibut in Myhre 1969; Takeuchi and Koike 1969; Kanda et al. 1978; Koike and Kanda 1978; Leclerc and Power 1980). However, in most of these studies the righthand "descending" limb of the selection curve was ill-defined due to sample size limitations.

In contrast, some investigators have been unable to show a decline in the righthand limb of hook selection curves, suggesting something akin to the asymptotic logistic selectivity of trawl gear (Pope et al. 1975). In particular, length–frequency samples of cod studied by McCracken (1963) and Saetersdal (1963) showed: (1) no difference from trawl catch size structure

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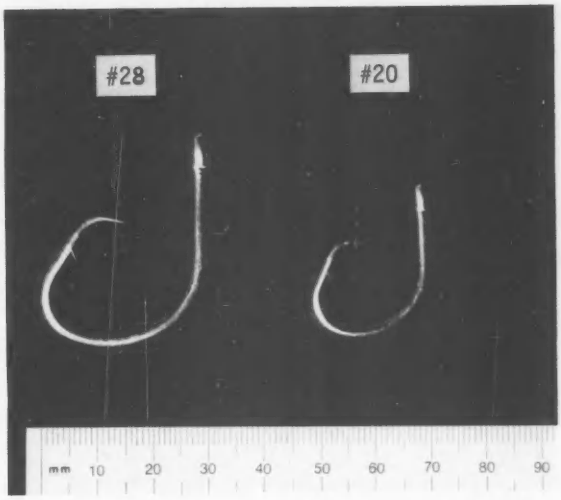

FIG. 1. The two sizes of Izuo circle fish hooks used during comparative fishing trials in the Marianas archipelago from 1982–84.

and (2) no effect of changing hook size on the catch of medium-large sized fish. Comparable findings were also reported for halibut by Myhre (1969); Ralston (1982); and Bertrand (1988).

The purpose of this study was, therefore, to perform a comparative fishing experiment (sensu Margetts 1969) with the goal of evaluating the efficacy of the logistic and normal selection models in describing patterns of hook selectivity.

Methods

As part of a multispecies resource assessment (Polovina and Ralston 1986), deepwater (100–300 m) fishing with hook-and-line gear was conducted throughout the Mariana Islands (lat. 13–20°N, long. 143–146°E) from April 1982 to May 1984. During this time six 40-d cruises were completed, with the waters surrounding all the islands of the archipelago sampled at least once, and some as many as six times (Polovina 1986). Fishing activity was directed toward the assemblage of eteline snappers (Lutjanidae), groupers (Epinephelinae), and jacks (Carangidae) that characteristically occupy deep reef habitats throughout the tropical Indo-Pacific (Forster 1984; Ralston et al. 1986).

Sampling was done from the R/V *Townsend Cromwell* while drift fishing with four hand-operated hydraulic fishing gurdies, each outfitted with ~365 m of braided prestretched Dacron line. The terminal rig was equipped with four Izuo[2] circle fish hooks (Forster 1973) attached by 60-cm gangions to the main line, which was weighted with a 2-kg iron bar. The two forward fishing lines on the vessel were equipped exclusively with #20 hooks, while the two aft lines only used #28 hooks (Fig. 1). Baits were pieces of uniformly cut stripped squid that were randomly allocated to each rig. Overall, the type and configuration of the fishing gear was similar to that employed commercially.

During fishing operations all four lines were fished concurrently so that at any point in time equal numbers (eight) of the two sizes of hooks were in use. All fish that were landed were classified by hook size of capture, identified to species, measured to the nearest millimetre fork length (FL), and weighed to the nearest 0.01 kg. Data were summarized for each species and hook size combination by rounding FL to the nearest 20 mm and aggregating the data into length–frequency distributions.

Evaluating Competing Models of Gear Selection

It is useful to draw a distinction between the intrinsic selective properties of a fishing gear and other aspects of "selectivity" extrinsic to the gear (Clark 1960). For example, year-to-year changes in the availability of a stock contribute to the latter. Likewise, selection curves obtained from cohort, virtual population, or catch-at-age analysis represent the combined effects of intrinsic and extrinsic selection factors. However, in this paper I refer to selectivity in the narrower, intrinsic sense, i.e., the size-specific likelihood of capture once a fish has encountered the gear. Selection coefficients are scaled to the fish size caught most readily and, therefore, range from 0.00–1.00. Thus, selection curves can be characterized for specific gear types, irrespective of changes in the distribution or behavior of the fish.

A more explicit quantitative definition of selectivity is provided by the relationship:

$$(1) \quad C_{i,j} = s_{i,j}\, q_i\, f_i\, N_j$$

where $C_{i,j}$ is the catch in numbers of fish in size class j on some fishing gear of size i; $s_{i,j}$ is the selection coefficient of gear size i to fish size j; q_i is the full selection ($s_{i,j} = 1.00$) catchability coefficient of gear size i; f_i is the amount of fishing effort with gear size i; and N_j is the number of fish in the population of size j (based on Hamley 1975).

A standard gear selection curve shows the relationship between the selection coefficient on the ordinate and fish size on the abscissa (Type A curve sensu Regier and Robson 1966). While there are a number of ways in which to estimate the shape of a selection curve, direct determination (Pope et al. 1975) is best. Unfortunately, there are few such studies in the literature, especially for hooks, because the size structure of the sampled population must be accurately known, a difficult requirement to meet. A more common practice is to compare the size structure of the catch made on different sizes of fishing gear to estimate the parameters of an assumed theoretical selection curve (e.g. Holt 1963), or to develop Type B selection curves (selection coefficient plotted against size of gear for a particular size class of fish).

In situations where the underlying form of the theoretical selection model is known or assumed (e.g. the logistic curve for trawl gear or the normal curve for gillnets), Type B curves have been used to estimate model parameters (see Hamley 1975 for a review of methods). In a more general context, however, comparative fishing studies provide little direct information about the shape or general form of an unknown selection curve (Pope et al. 1975). Still, comparative fishing can provide a basis for testing the validity of competing selection models.

If two different sizes of the same type of gear (e.g. hooks) are fished simultaneously on the same stock of fish with the same amount of fishing effort (i.e., $f_1 = f_2$ where the subscripts 1 and 2 refer to the smaller and larger gears, respectively), then as long as full vulnerability catchability is independent of gear size (i.e., $q_1 = q_2$), the ratio of catch in any common length class j ($C_{2,j}/C_{1,j}$) is equal to the ratio of selection coefficients

[2]Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

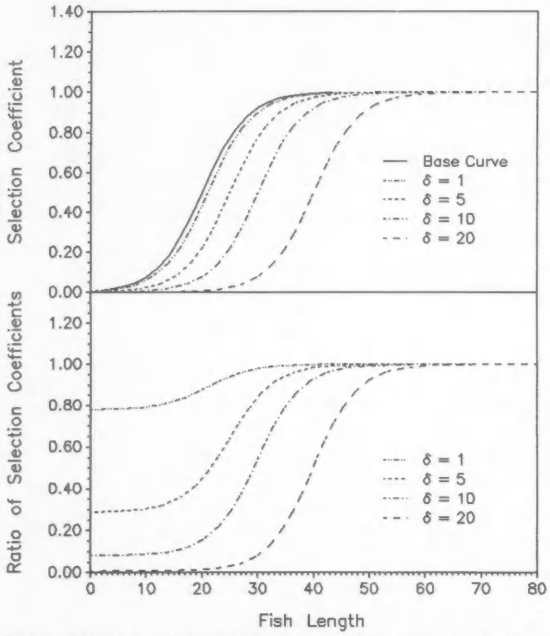

FIG. 2. Predicted ratios of catch taken on different sizes of a hypothetical fishing gear governed by logistic selection. See text for further explanation.

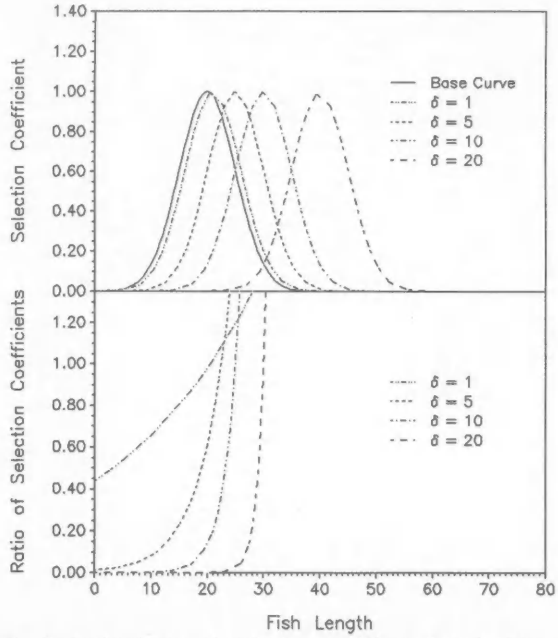

FIG. 3. Predicted ratios of catch taken on different sizes of a hypothetical fishing gear governed by normal selection. See text for further explanation.

($s_{2,j}/s_{1,j}$). This property can be used to test the hypothesis that hook selectivity is best described by either the logistic or normal curves.

Specifically, if selection by fish hooks, like trawls, is governed by the logistic curve (Pope et al. 1975), then the ratio of catches taken on different hook sizes when plotted against length interval should, like the ratio of two logistic curves with common asymptote, increase asymptotically to a value of 1.00 (Fig. 2). Note that in the upper panel of the figure the standard base curve (solid line) depicts the selection curve for the smallest gear size. The other four curves represent selection curves for larger sizes of the same type of gear. Increasing gear size is presumed to simply shift selection curves to the right, without altering the asymptote or rate (i.e., steepness) parameters. The degree of shift is measured by δ, i.e., the difference between the scaling/position parameters of the selection curve for the larger gear and the base curve. The lower panel depicts the ratio of selection coefficients as a function of length, for each of the shifted curves relative to the base curve. This ratio consistently approachs a value of one.

Alternatively, if selection by hooks is, like gillnets, governed by the normal curve (Hamley 1975), then the ratio of catches by length interval should, like the ratio of two normal curves with similar height and dispersion parameters, increase geometrically without bound (Fig. 3). As in the preceding example, the upper panel depicts a base selection curve for the smallest gear size (solid line), with four selection curves from larger gears shifted progressively to the right. With increasing size of gear there is no alteration in the height or dispersion parameters of the selection curves. The degree of shift is again measured by δ, the difference in scaling parameters between each curve and the base. The ratio of selection coefficients, for each of the

TABLE 1. Catch of four deepwater snappers by fork length category and hook size.

Fork length (mm)	*Pristipomoides zonatus*		*Pristipomoides auricilla*		*Pristipomoides flavipinnis*		*Etelis carbunculus*	
	#20	#28	#20	#28	#20	#28	#20	#28
200	2	1	0	0	0	0	0	0
220	3	2	1	0	0	0	2	0
240	9	5	2	0	0	0	1	2
260	27	23	21	5	0	0	5	4
280	46	48	36	11	0	0	16	13
300	92	84	97	30	2	2	30	41
320	138	169	142	44	7	4	35	41
340	181	226	157	50	15	11	69	95
360	236	334	169	62	25	19	80	76
380	280	405	115	41	51	51	63	83
400	228	379	50	24	54	44	25	56
420	175	255	3	2	20	34	12	19
440	61	88	0	0	15	24	7	17
460	2	9	0	0	7	6	2	7
480	0	1	1	0	0	3	2	2
500	0	1	0	0	1	1	0	1
520	0	0	0	0	0	0	0	0
540	0	0	0	0	0	0	1	0
Total	1480	2030	794	269	197	199	350	457
Species total	3510		1063		396		807	

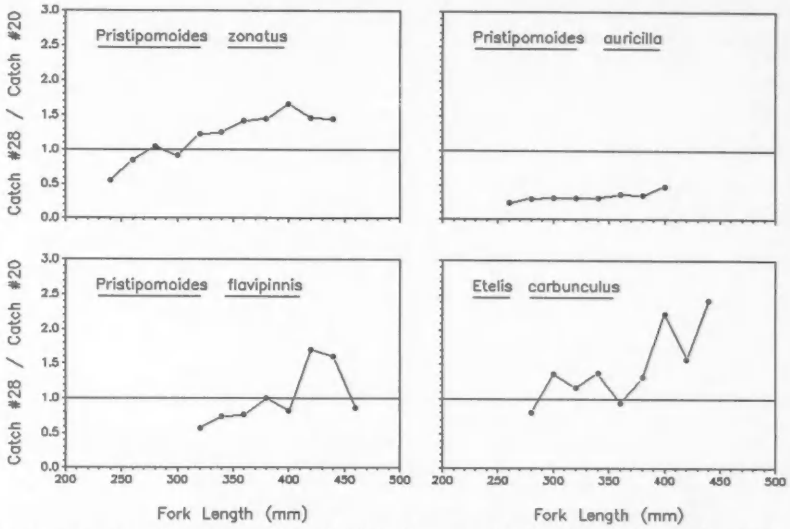

FIG. 4. Ratios of catches taken on #28 and #20 hooks, summarized by 20-mm FL categories, for the four species examined. A ratio of one corresponds to equal numbers of fish on the two hook sizes.

shifted curves relative to the base curve, is shown in the lower panel. In all cases the ratio of selection coefficients displays a precipitous monotonic increase.

Results

Over 7000 fish, comprising in excess of 30 species, were caught during handline fishing operations (see also Polovina 1986; Ralston and Williams 1988; Ralston 1988). However, 86% of the catch was composed of four snapper species: *Pristipomoides zonatus*, *P. auricilla*, *P. flavipinnis*, and *Etelis carbunculus*. Due to the relatively good catches of these four species, hook size catch ratios by length interval were calculated for each. Catch totals show (Table 1) that *P. zonatus* was particularly abundant, accounting for over half of all fish caught.

An examination of the size-specific ratio of catches taken on size #28 hooks, divided by the catches taken on size #20 hooks, shows a consistent increasing trend with FL for each species (Fig. 4). To reduce instability due to small sample size, catch ratios were calculated only if the combined sample size in a 20-mm interval of FL was ≥ 10.

Results for *P. zonatus* show that the smaller hooks caught more small fish than did the larger hooks (ratio <1.00). At a size of about 280 mm FL an equivalent number of fish were caught on each size of hook and at the larger sizes the largest hooks performed better. A Spearman rank correlation analysis of the *P. zonatus* data in Fig. 4 resulted in $r=0.927$ ($P=0.0001$), confirming the strong dependence between the size structure of the catch and the size of hook used.

Regardless of the size of fish, the smaller #20 hooks always caught more *P. auricilla* than did the larger #28 hooks, although to a lesser extent as fish size increased. Still, there was a strong Spearman rank correlation between fish size and the ratio of catch taken on the two hook sizes ($r=0.976$, $P=0.0001$). The low catch rate of *P. auricilla* on large hooks, in comparison to the other three snapper species, is likely due to its relatively small gape (Allen 1985) and planktivorous food habits (Seki and Callahan 1988).

For most sizes the catch of *P. flavipinnis* was generally greater on the small hooks than on the larger hooks, except in the largest length categories (FL >400 mm). Though not as strong as that of the preceding two species, a significant Spearman correlation existed between the size of *P. flavipinnis* and the ratio of catches taken on the size #28 and #20 hooks ($r=0.786$, $P=0.021$).

In contrast to the preceding two species, more *E. carbunculus* were caught on the #28 hooks than on the #20 hooks, especially in the largest size classes. As with all species examined, there was a significant Spearman rank correlation between fish size and catch ratio ($r=0.767$, $P=0.016$).

Discussion

The results presented here do not directly support either of the selectivity models presented earlier. The simple logistic model (Fig. 2) is not supported because the ratio of catches taken on the different hook sizes consistently rises to values in excess of 1.00. Likewise, the simple normal model (Fig. 3) is not supported because hook catch ratios do not seem to increase geometrically without bound.

Both these models are simple because full selection catchability is assumed to be unaffected by alterations in gear size (i.e., $q_1 = q_2$). There is evidence to show, however, that the catchability of optimally sized walleye to gillnets increases with increasing mesh size (Hamley and Regier 1973; Hamley 1975). Similarly, Beverton and Holt (1957) present data from alternating haul experiments showing that large mesh trawls catch more plaice in shared fully vulnerable size categories than do small mesh trawls, suggesting an increased efficiency of large mesh gear.

These findings demonstrate that increasing gear size can lead not only to a righthand shift of the selection curve, but also to an increase in catchability. Thus, the assumption that catcha-

bility in the fully vulnerable size range is independent of gear size is too simplistic.

If catchability varies the catch ratio is no longer equal to the simple ratio of selection coefficients, but includes the ratio of catchability coefficients as an additional term; that is:

$$C_{2,j}/C_{1,j} = (s_{2,j}/s_{1,j})(q_2/q_1)$$

Extending this result to a hypothetical model of logistic hook selection would suggest that catch ratios can exceed a value of one if $q_1 < q_2$. Catch ratios would then asymptotically approach a value equal to the ratio of catchability coefficients, rather than approaching a value of 1.00. The results presented in Fig. 4 (especially *P. zonatus*) are consistant with this interpretation, but they are not conclusive.

Conversely, a hypothetical model of hook selection based on righthand shifted normal curves in conjunction with progressively increasing full selection catchability coefficients, would simply accentuate the geometric unbounded increase evident in Fig. 3. Although none of the results presented in Fig. 4 seem to conform to such a pattern, it could be that the size range of fish sampled was too small to detect the strong curvature predicted by the model. Such an explanation, however, seems unlikely.

Our present understanding of the intrinsic nature of hook selectivity is rudimentary at best. There is no generally accepted theoretical model of selection by fish hooks, as there are for trawls, gillnets, and other meshed gears. It is likely that in reality neither of the models examined is particularly well-suited to the quantitative description of hook selectivity, although of the two, the logistic model is most consistent with the comparative fishing results presented here (see also Ralston 1982). What is needed is a direct determination (Pope et al. 1975) of selection coefficients through an experimental program, in which a population of known size structure is fished with hooks of various sizes. The sampled population could either be experimentally fished in captivity or it could be a known marked population in the field. The latter approach has been used effectively with gillnets (Hamley and Regier 1973) and was employed with limited success by Leclerc and Power (1980) in their work on angling and gillnet selectivities. In their study, however, small sample sizes prevented precise resolution of the selection pattern. Likewise, in two separate studies, Myhre (1969) compared the size structure of Pacific halibut captures with hooks against a known marked population, but he obtained conflicting results. Regardless, until a more definitive study is completed, it will be difficult to make inferences about the size structure of fish populations from single catch samples acquired with hook-and-line gear.

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Reconditioning and Reproductive Responses of Atlantic Salmon Kelts (*Salmo salar*) to Photoperiod and Temperature Manipulation

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The reproductive cycle of reconditioned Atlantic salmon can be manipulated with light and temperature. Wild Atlantic salmon (*Salmo salar*) kelts held in freshwater and exposed from late December 1986 to a simulated natural photoperiod (*NP*) and a regime of two 6-mo seasonally accelerated light increases and decreases (2*CP*) reconditioned. Fifty-six percent of *NP* females spawned by early November 1987, 1 mo later than virgin wild females. No 2*CP* females spawned by December 1987. However, all 2*CP* females transferred in December 1987 to natural photoperiod and 7°C water released eggs by June 1988. *NP* females that spawned showed seasonal peaks in cholesterol in August, triglycerides and calcium in September, and alkaline phosphatase in November; *NP* and 2*CP* females that failed to spawn showed no such changes. Alkaline phosphatase activity and calcium concentrations were linearly related to increasing oocyte diameter (but not oocyte number), and were good predictors of sex and oocyte development. Forty-two percent of 2*CP* males matured and released sperm in mid-August, 1 mo earlier than *NP* males or a virgin wild group. Twenty-three percent of 2*CP* males did not mature. Males showed significant differences in blood parameters between regimes and between spawners and non-spawners, but the levels were never as high as maturing females.

Il est possible d'intervenir sur le cycle reproducteur du saumon de l'Atlantique (*Salmo salar*) revigoré après la ponte en jouant sur l'éclairement et la température. Des charognards sauvages maintenus en eau douce et exposés à partir de la fin de décembre 1986 à une photopériode naturelle simulée (*NP*) et à un régime de deux périodes de 6-mo d'augmentation et de diminution accélérées de l'éclairement (2*CP*) se sont revigorées. Parmi les femelles *NP*, 56 % avaient pondu au début de novembre 1987, 1 mo plus tard que les femelles vierges sauvages. Aucune des femelles 2*CP* n'avait pondu en décembre 1987. Cependant, toutes les femelles 2*CP* transférées en décembre 1987 dans de l'eau à 7°C en photopériode naturelle avaient pondu en juin 1988. Les femelles *NP* qui ont pondu ont présenté des pics saisonniers de cholestérol en août, de triglycérides et de calcium en septembre, et de phosphatase alcaline en novembre; les femelles *NP* et 2*CP* qui n'ont pas pondu ne présentaient pas ce type de modification. L'activité de la phosphatase alcaline et les concentrations de calcium présentaient une relation linéaire avec l'augmentation du diamètre des ovocytes (mais non avec le nombre des ovocytes), et constituaient de bons prédicteurs du sexe et du développement des ovocytes. Parmi les mâles 2*CP*, 42 % avaient atteint la maturité sexuelle et frayé à la mi-août, 1 mo plus tôt que les mâles *NP* ou que ceux du groupe vierge sauvage; 23 % des mâles 2*CP* n'ont pas atteint la maturité. Les mâles montraient des différences notables dans les paramètres sanguins selon le régime et selon qu'ils étaient ou non reproducteurs, mais ces chiffres n'étaient jamais aussi élevés que chez les femelles en cours de maturation.

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Atlantic salmon (*Salmo salar*) spawn in the fall and unlike most Pacific salmon and trout (*Oncorhynchus* spp.) are iteroparous; thus capable of spawning several times during their lifespan. While the proportion of repeat spawners in the wild can vary considerably among rivers, generally only 5–10% return to spawn a second time and less than 1% a third time (Gray et al. 1976). The failure of salmon to return in larger numbers may be related in part to commercial exploitation or to their weakened body condition and depleted body reserves after spawning and overwintering. Poor body condition may make them more susceptible to disease or more vulnerable to predators while undergoing seaward or marine migrations. The loss of so many salmon in the wild can be reduced by retaining adults after spawning and reconditioning them in captivity. In a recent study, we demonstrated that 80–95% of wild Atlantic salmon kelts taken into captivity can be successfully reconditioned in freshwater (Johnston et al. 1987). These results are similar to several earlier studies (Ducharme 1972; Gray et al. 1976; Hill 1978; Gray et al. 1987). However, while it is possible to recondition kelts successfully in freshwater, only 40% of the females successfully mature and produce eggs in the first year in captivity. Failure to attain 100% maturation of the females may be related to stress associated with captivity, an insufficient level of body reconditioning (low weight gain and low condition

factor) at the time of vitellogenesis, or inappropriate environmental cues to stimulate hormonal release governing oogonial proliferation, vitellogenesis, and oocyte maturation.

Environmental cues such as photoperiod and temperature have been shown to affect the qualitative and quantitative components of reproduction in Atlantic salmon (Johnston et al. 1987). They influence either directly or indirectly the level of food consumption, rate of tissue rehabilitation after spawning, egg quality, egg quantity, rate of gonad rematuration, and time of spawning. In other studies, photoperiod manipulation has been used to accelerate gametogenesis and spawning in rainbow trout (*Oncorhynchus mykiss*) (Whitehead et al. 1978; Bromage et al. 1982a,b; Duston and Bromage 1987, 1988). By compressing a normal yearly cycle into 6 mo the reproductive cycle of rainbow trout can be advanced 12 wk (Whitehead et al. 1978; Duston and Bromage 1986). Photoperiod appears to be the more important determinant for salmonids, with spawning times either advanced or retarded with the light cycle. However, it is not known if photoperiod conditions alone or in some special combination with temperature must be used to advance or improve the rematuration process in wild Atlantic salmon kelts held in captivity. The physiological responses to photoperiod and temperature manipulation remain poorly described for reconditioned male and female salmon. Knowledge of the physiological responses could be useful in improving the reconditioning response, the number of males or females maturing, or the time of maturation. With reductions in wild salmon populations and increased demand for eggs in aquaculture there is now great interest in rehabilitating wild salmon following spawning. Improvements in the rematuration process could be of considerable advantage to hatcheries attempting to use wild kelts for broodstock.

In the present study, male and female Atlantic salmon kelts were exposed to a simulated natural and compressed yearly light cycles (*2CP*) to determine if the level of reconditioning and rematuration responses could be improved and spawning times shifted. We chose two 6-mo light cycles for testing because these conditions were effective in stimulating early maturation in rainbow trout (Bromage et al. 1982a). Furthermore, if oocytes failed to develop in the first compressed cycle then the second cycle should be stimulatory.

Seasonal changes in plasma alkaline phosphatase activity, cholesterol, triglyceride, and calcium concentrations together with gonad state were measured to provide physiological information on the differences in the timing and the rates of rematuration for both sexes. Since our earlier study (Johnston et al. 1987) showed that females displayed seasonal patterns of change in these parameters with clear differences between spawners and nonspawners we wanted to establish whether there were similar blood chemistry changes in males.

Materials and Methods

During the summer of 1986, wild male and female early run Atlantic salmon were captured in a trap on the Morell River, Prince Edward Island and transported to the Cardigan Fish Culture Station where they were held until spawning and the start of this experiment. The captured salmon were from Miramichi River stock introduced into the Morell River in 1985. In December, 46 postspawned females and 30 males were anaesthetized with tricaine methane sulfonate (0.1 $g \cdot L^{-1}$) individually identified with Carlin tags, weighed, measured for fork length and randomly placed in two, 3-m center-draining fiber-

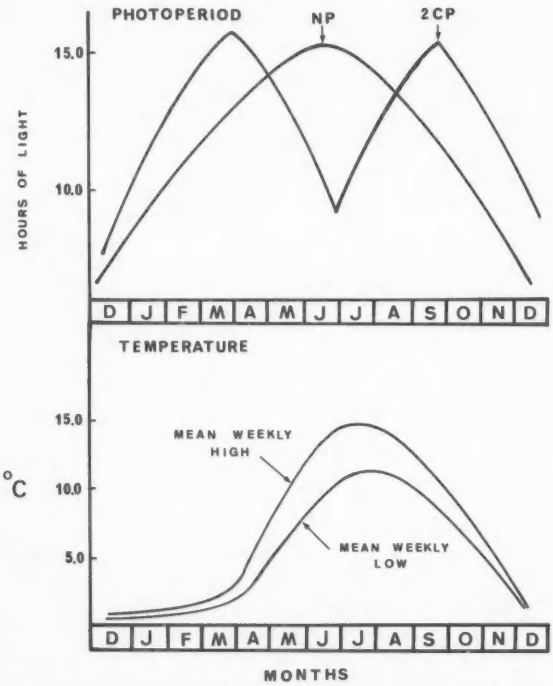

FIG. 1. Summary of natural (NP) and compressed 6 month light (2CP) cycles and mean weekly water temperatures supplied to each experimental regime.

glass Swede tanks (approximate volume 9 m^3) that received approximately 38 $L \cdot min^{-1}$ of Cardigan River water. The design of the tanks, lighting specifications, and aeration system were the same as described earlier by Johnston et al. (1987).

Commencing on 21 December 1986 each tank was placed on a different photoperiod regime (Fig. 1). The first tank or control group (*NP*) was exposed to a simulated natural photoperiod cycle where daylength increased and decreased naturally over a 12-mo period. The length of the daily light period was controlled by a photocell situated outdoors. To test whether reconditioning and maturation could be accelerated, a two-cycle photoperiod group (*2CP*) was given two cycles of 6-mo accelerated natural photoperiod, compressing the annual light cycle. The first 6-mo cycle was initiated on 21 December and the hours of light were increased to the longest day in late March and then decreased to the shortest day in July. In the second cycle the hours of light increased from July until the longest day in late September and then decreased to the shortest day in December. The length of the daily light period was controlled by a timer manually set each week. The length of the daily light period and photocycle in each tank was monitored and verified using a continuous recorder. Each tank was supplied with water from the Cardigan River; water temperatures followed daily and seasonal fluctuations (Fig. 1). One year later, on 23 December 1987, *2CP* and nonspawning *NP* females were taken off the light and temperature regimes and placed on 7°C well water and a simulated natural photoperiod cycle until 23 March 1988. They were fed a moist pellet diet. On 23 March they were weighed, measured for fork length, checked for reproductive maturity, and transferred to an outdoor pond where they were exposed to natural light conditions and 4–6°C water tempera-

TABLE 1. Summary of the foods used to feed salmon during the experimental period.

Date	Food type	Source
4 February–8 May	Immature smelts (*Osmerus mordax*)	Commercial fishermen in PEI
21 April–9 July	Mature smelts (male and females)	Collected in a river just before spawning
10 July–23 September	Immature capelin (*Mallotus villosus*)	Commercial fisherman in Newfoundland

tures until late April. The warmer outdoor water temperatures were achieved by mixing equal volumes of Cardigan River water with 7°C well water. In late April as water temperature rose above 6°C the use of well water was terminated and salmon were placed on river water. Water temperatures during May and early June were similar to those shown in Fig. 1. On 10 June salmon were again weighted, measured for length, and checked for maturity.

Reconditioning

Commencing in January 1987, salmon were force-fed with freshly thawed silversides (*Menidia menidia*). This was accomplished using a long pole with a nail on the end. The 2-cm projecting nail was forced into the anus and body cavity of the silverside so that the head of the silverside could be inserted into the mouth of the salmon. The long pole enabled placement of the silverside in the mouth of each salmon from a distance. Silversides placed in the mouth this way were usually consumed. Occasionally, salmon would reject the food and only after repeated offerings would feeding commence. Once a strong feeding response was developed, the force-feeding method was discontinued and the silversides were thrown into the water near the salmon. Most salmon in both regimes were feeding to some extent by February. Due to a limited supply of silversides, a variety of foods were used during the experimental period (Table 1). Prior to each feeding silversides, smelts (*Osmerus mordax*), and capelin (*Mallotus villosus*) were injected with a mineral and vitamin mix (Table 2).

Blood Plasma and Egg Measurements

Blood samples were first taken from all salmon in December 1986 and at approximately monthly intervals thereafter. Condition factor was calculated using the equation (weight/fork length^3) × 100. Prior to each sampling salmon were starved for at least 24 h. Blood samples were drawn from the caudal vein into lithium heparinized syringes and immediately placed on ice. Within 1–2 h blood was centrifuged at 3000 RPM and plasma separated from the red blood cells. Samples of blood were also measured for hematocrit to evaluate the effect of repeated monthly blood sampling on the volume of leucocytes and red blood cells. Plasma was immediately assayed in duplicate for alkaline phosphatase activity (*AP*), cholesterol, triglyceride, and calcium concentrations. Reagents for each assay were prepared by Diagnostic Chemicals Limited, Charlottetown, P.E.I. and assays were conducted according to the described protocol.

During the experimental period female salmon were killed periodically to evaluate ovarian development. Prior to killing, blood samples were taken and evaluated for blood chemistry parameters. Ovaries were removed, weighed, measured for volume by volumetric displacement methods, and dissected for total oocyte count and oocyte diameter measurements with a calibrated microscope. Egg counts and diameters were also measured at spawning. This was accomplished by stripping and fertilizing the eggs, water hardening them for 3 h and disinfecting them for 10 min in a 10% solution of Ovadine (1.04% free iodine) before measuring egg number and diameters. Egg number was determined by the displacement method (Burrows 1951) and egg diameters were measured in a 15-cm egg trough (von Bayer 1910).

TABLE 2. Mineral and vitamin levels injected into each smelt or capelin prior to feeding it to salmon.

Item	Milligrams per fish
Minerals	
NaCl	15
Potassium iodide	0.06
Manganous sulfate	1.4
Copper sulfate	0.35
Zinc sulfate	2.64
Magnesium sulfate	4.86
Water-soluble vitamins	
Thiamine	0.03
Riboflavin	0.06
Pyridoxine	0.03
Pantothenic acid	0.12
Biotin	0.003
Vitamin B_{12}	0.00006
Niacin	0.0004
Ascorbic acid	0.3
Folic acid	0.02
Inositol	1.2
Fat-soluble vitamins	
Vitamin A	85 IU
Vitamin D_3	8.5 IU
Vitamin E	80 IU
Vitamin K	0.18
Carophyll red (10%)	0.2

A third group of wild salmon was caught in a trap as the fish ascended the Morell River in July and August 1987. They were transported to the Cardigan Fish Culture Station and held indoors. This group was used to evaluate the level of blood parameters and ovary development in fish exposed to natural light, temperature, and feeding conditions.

Statistics

The numbers of salmon sampled from each regime during the experimental period are shown in Table 3. Differences between means of the parameters measured were tested statistically using a Duncan's multiple range test or a Student's *t*-test.

Results

Reconditioning Responses

Salmon began feeding in January 1987 when water temperatures were between 0–2°C. Generally, food consumption increased slowly in both regimes until water temperatures rose in April. Thereafter a strong feeding response developed and persisted until mid-August when food consumption declined. While there was a slight decline in 2*CP*

TABLE 3. Number of salmon sampled for each regime, December 1986–December 1987. S = spawning, M = male, NP = natural photoperiod, NS = nonspawning, F = female, *2CP* = two cycle photoperiod.

	Dec.	Feb.	Apr.	May	July	Aug.	Sept.	Oct.	Nov.	Dec.
S F NP	8	8	8	7	8	8	8		8	7
NS F NP	15	14	15	12	10	10	9			7
NS F *2CP*	23	23	23	21	20	21	21			21
S M NP		15	14	12	11	12	12			11
S M *2CP*		10	10	10	10	10	10			10
NS M *2CP*		5	5	3	3	3	3			2
Wild male grilse					12	10	10	0		
Wild female grilse					4	10	9	9		

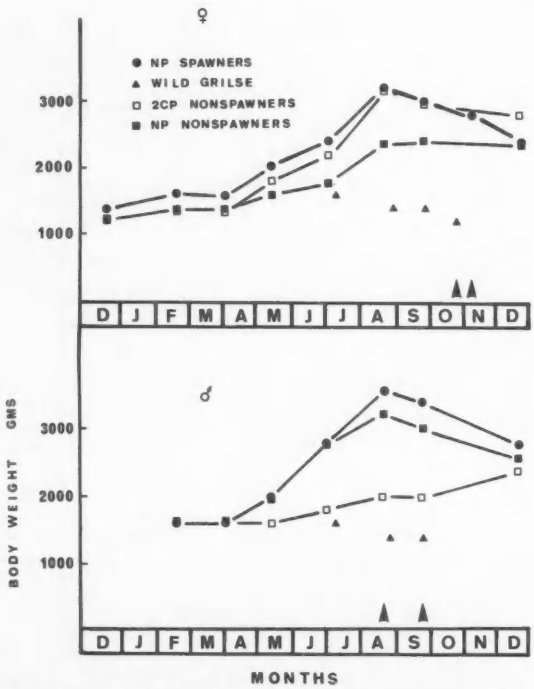

FIG. 2. Seasonal changes in mean body weight for spawning and nonspawning female and male Atlantic salmon kelts exposed to natural photoperiod (NP) and compressed 6 month light (2CP) regimes, and for wild female and males grilse captured in the Morell River and held under natural light conditions. Arrows indicate time of spawning for female wild grilse (Oct), and salmon in NP (Nov) and time of spermiation for male salmon in 2CP (Aug) and NP (Sept).

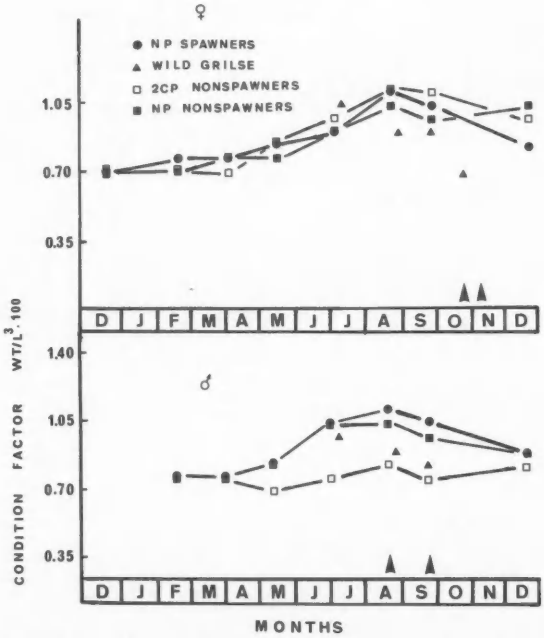

FIG. 3. Seasonal changes in condition factor for spawning and nonspawning female and male Atlantic salmon kelts exposed to natural photoperiod (NP) and compressed 6 month light (2CP) regimes, and for wild female and male grilse captured in the Morell River and held under natural light conditions. Arrows indicate time of spawning for female wild grilse (Oct) and salmon in NP (Nov) and time of spermiation for male salmon in 2CP (Aug) and NP (Sept).

feeding in late August, *2CP* females continued to display a stronger feeding response than *NP* fish and this response persisted until March.

Most groups of fish showed weight gains between April and August (Fig. 2). Exceptions occurred among female *NP* and male *2CP* nonspawners which were significantly ($p<0.05$) lighter than either *NP* or *2CP* spawners. Weight losses occurred between August and December and were due to spawning and lack of feeding. Similar weight losses did not occur for nonspawning females. Mean weight gains and losses for spawning males in *NP* and *2CP* were similar, but nonspawning males had significantly lower ($p<0.05$) weights in August and September.

Seasonal patterns of change in mean condition factor for males and females were similar to the changes observed for mean fresh weights (Fig. 3). Males with a condition factor between 0.70 and 0.75 in September did not develop mature gonads. Photoperiod manipulation did not accelerate the reconditioning process.

Rematuration Responses

All of the wild grilse and only 56% of the *NP* female kelts spawned in October and early November; no *2CP* females spawned by the end of December 1987 (Table 4). Egg release occurred on 24 October for six of the nine wild grilse and on 7 November for seven of the eight *NP* salmon with the final female maturing on 19 November. Egg diameters and fecundity

TABLE 4. Sample number, total mortality (sampling and natural), and percent maturing by December 1987 in both regimes and in wild grilse.

	Regime					
	NP[a]		2CP[b]		Wild Grilse	
	F[c]	M[d]	F	M	F	M
Initial sample No.	23	15	23	15	10	10
Total mortality	9	4	2	3	1	0
Percent maturing by December 1987 (mortality excluded)	56	100	0	77	100	100

[a]*NP* natural photoperiod.
[b]*2CP* compressed 6-mo light cycles.
[c]F = female.
[d]M = male.

TABLE 5. Egg diameters and fecundity for natural photoperiod (*NP*) and wild grilse spawners. Mean fork length and range for each group are shown. NP = natural photoperiod regime, SE = standard error of the mean.

	NP[a] spawners (Nov. 7/88)		Wild grilse spawners (Oct. 24/88)	
	Egg diameter	Egg No.	Egg diameter	Egg No.
	0.508	6656	0.554	3384
	0.566	6471	0.526	2031
	0.454	3947	0.528	2230
	0.583	4551	0.541	2540
	0.494	4605	0.533	2322
	0.500	5599	0.537	2667
	0.513	4291		
	0.503	4419		
Mean	0.515	5067	0.537	2529
SE	0.014	367	0.004	194
Mean fork length (cm)		65.9		54.3
Range		61.5–73.4		49.5–58.0

for spawning females varied (Table 5). Mean egg diameter although larger in wild grilse was not significantly different, but the mean egg number for *NP* spawners was significantly greater ($p<0.05$) than wild grilse. Also the mean egg number per centimetre fork length was significantly greater ($p<0.05$) for *NP* salmon (76.9) than wild grilse (46.6).

By late December 1987, females in the *2CP* regime had not spawned although there was some evidence from the blood chemistry data and increase in oocyte size (3 mm) in those sampled to suggest that vitellogenesis was proceeding. Because of the very low Cardigan River water temperatures in the winter, the females were placed on 7°C well water and a simulated natural photoperiod from late December to 23 March. At this temperature females continued to feed and were fed a moist pellet diet in place of whole capelin. Females were not handled again until late March when they were checked for maturity. At this time, females showed no external signs (protruding vents or bagging-up) of maturing. However, one female that died in March had ovaries with oocyte diameters ranging between 3.5–4.2 mm. Females were then transferred to an outdoor pond where they were exposed to an increasing natural light regime and water temperatures that varied between 4 and 6°C until the end of April followed by natural river water temperatures until 10 June. On 10 June females were again checked for maturity. At this time, all *2CP* females had matured and eggs could be

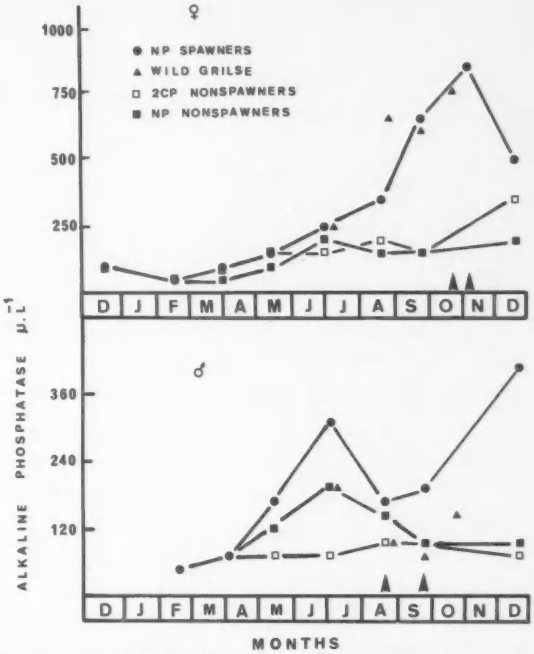

FIG. 4. Seasonal changes in plasma alkaline phosphatase (AP) activity for spawning and nonspawning female and male Atlantic salmon kelts exposed to natural photoperiod (NP) and compressed 6 month light (2CP) regimes, and for wild female and male grilse captured in the Morell River and held under natural light conditions. Arrows indicate time of spawning for wild female grilse (Oct) and salmon in NP (Nov) and time of spermiation for male salmon in 2CP (Aug) and NP (Sept).

released with sight pressure. Egg diameters ranged between 4.5 and 5.5 mm and were similar in size to eggs released in the fall by *NP* females. *NP* females that did not spawn in the fall were exposed to the same conditions as the *2CP* females but they did not mature in the spring. Blood chemistry values (*AP* = 111–154 IU; calcium = 2.4–3.0 mmol/L; cholesterol = 4.9–6.4 mmol/L; triglycerides = 2.1–2.6 mmol/L) for the *2CP* females in June indicated a postspawned condition while *NP* females (*AP* = 383–452 IU; calcium = 3.4–3.5 mmol/L; cholesterol = 7.6–11.4 mmol/L; triglycerides = 3.67–10.2 mmol/L) were beginning the vitellogenic phase.

Forty-two percent of the males from the *2CP* regime were ripe and released sperm by August, 1 mo in advance of *NP* fish confirming our hypothesis. By December 1987, 77% of the *2CP* males and 100% of both *NP* and wild grilse matured and released sperm (Table 3). Males that matured, reconditioned more quickly than females or males that did not mature. Males that had a condition factor of 0.70–0.75 did not mature and spermiation only occurred in salmon that had a condition factor greater than 1.00. There was also little difference in the rate of reconditioning between maturing *2CP* and *NP* males, yet there was a marked difference in time of spermiation between the two groups.

Blood Chemistry Changes

Plasma alkaline phosphatase activity (*AP*) for females ranged between 75–150 units per litre from December to mid-May (Fig. 4). Between May and November, there was a significant rise

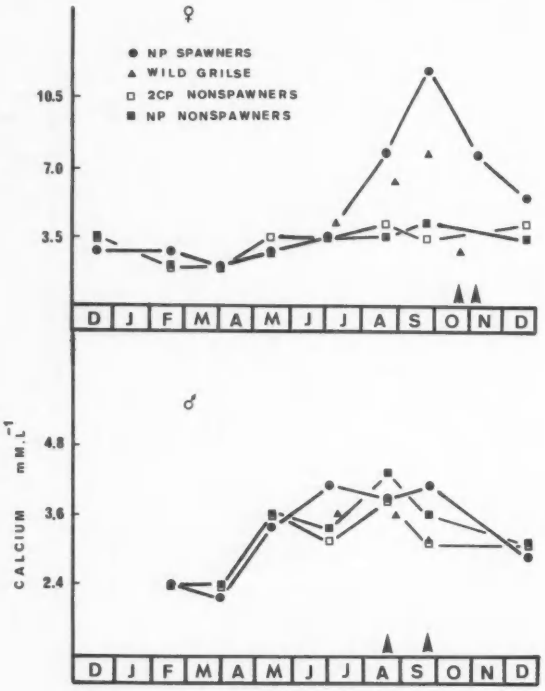

FIG. 5. Seasonal changes in plasma calcium concentrations for spawning and nonspawning female and male Atlantic salmon kelts exposed to natural photoperiod (NP) and compressed 6 month light (2C)P regimes, and for wild female and male grilse captured in the Morell River and held under natural light conditions. Arrows indicate time of spawning for wild female grilse (Oct) and salmon in NP (Nov) and time of spermiation for male salmon in 2CP (Aug) and NP (Sept).

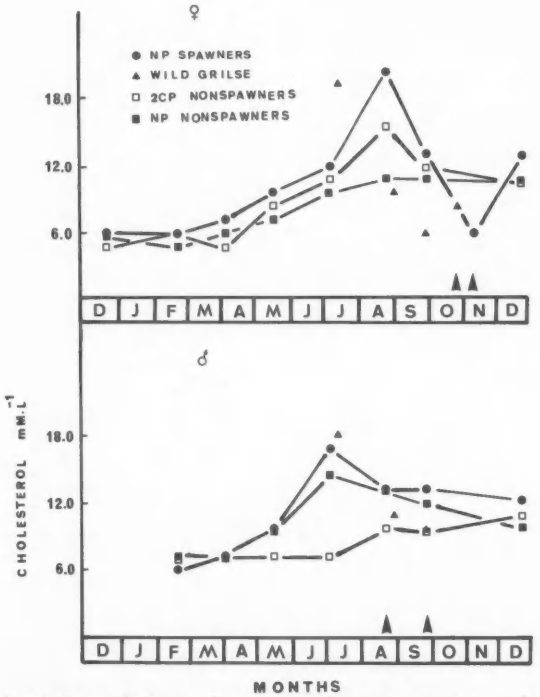

FIG. 6. Seasonal changes in plasma cholesterol concentrations for spawning and nonspawning female and male Atlantic salmon kelts exposed to natural photoperiod (NP) and compressed 6 month light (2CP) regimes, and for wild female and male grilse captured in the Morell River and held under natural light conditions. Arrows indicate time of spawning for wild female grilse (Oct) and salmon in NP (Nov) and time of spermiation of male salmon in 2CP (Aug) and NP (Sept).

($p<0.05$) in *AP* from 200 to 800 units per litre in *NP* spawning females. The *NP* levels during this period were similar to wild grilse females. Also the levels for *NP* spawners in September and November were significantly ($p<0.05$) greater than the levels for *NP* or *2CP* nonspawners. The *AP* increase for spawners coincided with vitellogenin synthesis and oocyte enlargement. Final oocyte maturation and release occurred for wild grilse in late October and for *NP* females in early November. Following egg release the *AP* level declined and by December was not significantly different from the other groups. However, in December some of the *2CP* nonspawners showed rising *AP* levels. As a result, several females were killed and the ovaries removed for oocyte evaluation. Oocyte diameters were 3 mm indicating oocyte enlargement when compared with the 1-mm diameter size in September. This response suggested that the second 6-mo light cycle was stimulating reproductive function in some of the females.

Except for *2CP* male nonspawners, *AP* levels in spawning males increased significantly ($p<0.05$) between February and July with levels being significantly higher ($p<0.05$) in *NP* and *2CP* spawners than *2CP* nonspawners (Fig. 4). Levels then declined and remained low in all groups during spermiation. An unusual and unexplained rise in *AP* levels occurred in *NP* spawners in December and this level was significantly higher ($p<0.05$) than in the other groups.

Plasma calcium levels for all females significantly increased ($p<0.05$) from April to August (Fig. 5). Females that spawned in *NP* displayed a significant peak ($p<0.05$) in September and thereafter a steady decline until December. The increase coincided with the stage of heightened exogenous vitellogenesis and oocyte enlargement. The increases in August and September were also significantly higher ($p<0.05$) than levels for *2CP* and *NP* nonspawners.

Males in all regimes showed significant increases ($p<0.05$) in calcium levels between February and July when food consumption increased (Fig. 5). Levels declined from August to December as water temperatures declined.

Plasma cholesterol levels for females in both regimes showed significant increases ($p<0.05$) from April to August (Fig. 6). Cholesterol levels of females that spawned in *NP* peaked in August and were significantly higher ($p<0.05$) than *NP* nonspawners. Cholesterol levels in wild females sampled in July were not significantly different than *NP* spawners in August. Levels declined significantly ($p<0.05$) in both groups as food consumption dropped and oocytes matured.

Cholesterol levels in male spawners rose steadily during the spring and coincided with increased food consumption. Levels peaked in July and were significantly higher ($p<0.05$) than *2CP* nonspawners (Fig. 6). Levels subsequently declined during spermiation and were not different thereafter between the groups.

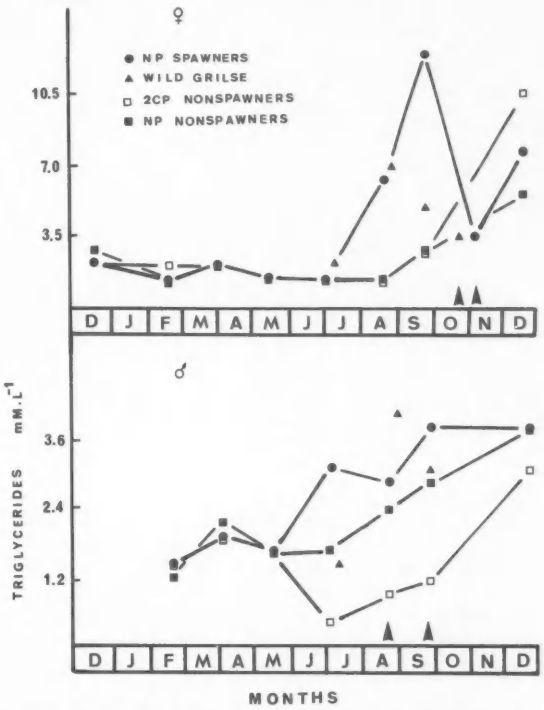

FIG. 7. Seasonal changes in plasma triglyceride concentrations for spawning and nonspawning female and male Atlantic salmon kelts exposed to natural photoperiod (NP) and compressed 6 month light (2CP) regimes, and for wild female and male grilse captured in the Morell River and held under natural light conditions. Arrows indicate time of spawning for wild grilse (Oct) and salmon in NP (Nov) and time of spermiation for male salmon in 2CP (Aug) and NP (Sept).

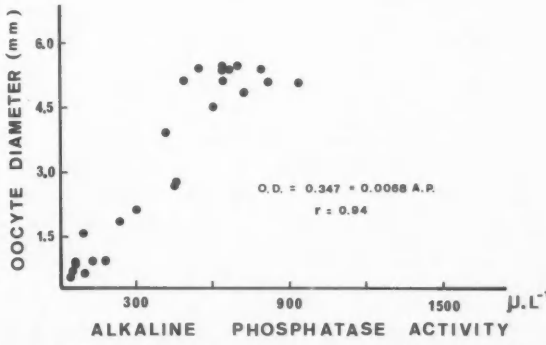

FIG. 8. Relationshp of oocyte diameter to plasma akaline phosphatase activity in female Atlantic salmon exposed to natural photoperiod (NP) and compressed 6 month light (2CP) regimes. The regression equation and correlation coefficient (r) for the points are shown.

Plasma triglyceride levels for spawning females in *NP* increased significantly ($p<0.05$) from July to September (Fig. 7). The September level was significantly higher ($p<0.05$) than any of the other groups, but declined during final oocyte maturation and release. Triglyceride levels between August and October in wild females also significantly ($p<0.05$) declined. No

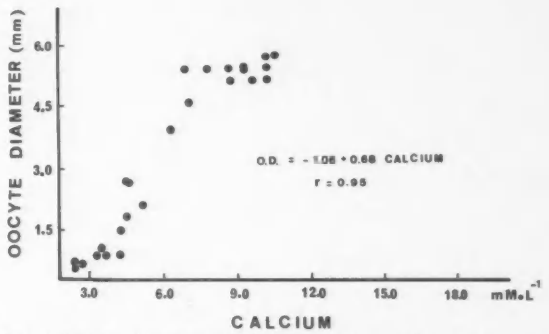

FIG. 9. Relationship of oocyte diameter to plasma calcium concentrations in female Atlantic salmon exposed to natural photoperiod (NP) and compressed 6 month light (2CP) regimes. The regression equation and correlation coefficient (r) for the points are shown.

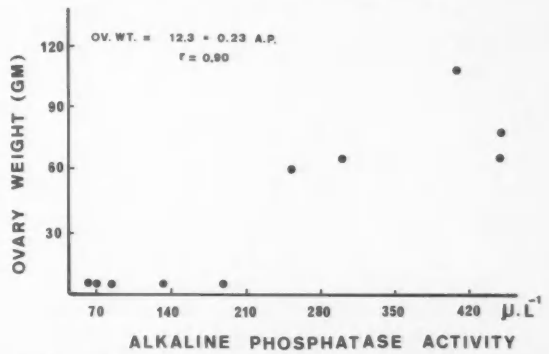

FIG. 10. Relationship of ovary weight to plasma alkaline phosphatase activity in female Atlantic salmon exposed to natural photoperiod (NP) and compressed 6 month light (2CP) regimes. The regression equation and correlation coefficient (r) for the points are shown.

similar change in triglyceride levels occurred in *2CP* nonspawners although a significant ($p<0.05$) increase in levels occurred by December. This increase was associated with an enlargement of oocytes in the ovary.

Triglyceride levels for all spawning males increased significantly ($p<0.05$) from July to December with levels being significantly greater ($p<0.05$) in July for the *NP* group (Fig. 7). Triglyceride levels for nonspawning males were significantly lower ($p<0.05$) than maturing males in July, August, and September. Triglyceride levels were not altered by photoperiod conditions, gonad maturation, or water temperature, but were affected by diet.

Oocyte Relationships to Blood Chemistry Parameters

A comparison of oocyte numbers and diameters with blood plasma chemistry levels revealed no correlation between oocyte number and any of the blood parameters. However, there was a highly significant relationship between oocyte diameter and alkaline phosphatase activity ($r=0.94$), and oocyte diameter and calcium concentration ($r=0.95$) (Figs. 8 and 9). There were weaker correlations between oocyte diameter and cholesterol ($r=0.88$), and oocyte diameter and triglyceride concentration ($r=0.64$). Oocyte weights were highly correlated with alkaline phosphatase ($r=0.90$) and oocyte diameter ($r=0.97$) (Figs. 10

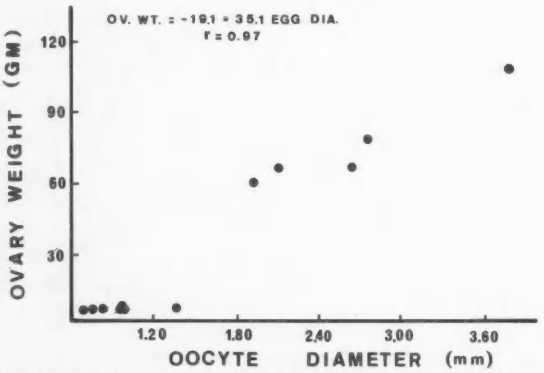

FIG. 11. Relationship of ovary weight to oocyte diameter in female Atlantic salmon exposed to natural photoperiod (NP) and compressed 6 month light (2CP) regimes. The regression equation and correlation coefficient (r) for the points are shown.

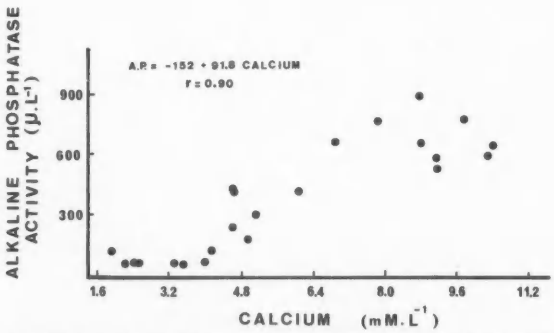

FIG. 12. Relationship of plasma alkaline phosphatase activity to plasma calcium concentrations in female Atlantic salmon exposed to natural photoperiod (NP) and compressed 6 month light (2CP) regimes. The regression equation and correlation coefficient (r) for the points are shown.

and 11). In addition, increases in *AP* activity were associated with increases in calcium concentration ($r = 0.90$) (Fig. 12).

Discussion

The present study demonstrates that it is possible to alter the spawning time of reconditioned wild Atlantic salmon with light and temperature manipulation. Some males matured earlier in the *2CP* regime and were stimulated by photoperiod manipulation. However, the *2CP* regime did not stimulate the females to recondition faster or mature earlier during the first year, but the light cycles did trigger the rematuration process by December and egg release by June of the second year. This is the first documented case of spring spawning by Atlantic salmon and it indicates that the annual cycle of fall spawning can be altered to obtain eggs at different times in the year.

The ability to mature Atlantic salmon at any time in the year could be useful to those in aquaculture. This combined with the cryopreservation of sperm and a year-round supply of eggs could greatly enhance salmon production for the future. For example, spawning salmon in the spring and hatching eggs when water temperatures are most desirable for early development could greatly reduce the overwinter losses of eggs, alevins, or fry. This could represent a considerable saving for many commercial and government-operated hatcheries and could possibly lead to producing 0+ smolts in Maritime hatcheries.

It has been shown that by compressing a normal yearly light cycle into 6 mo the reproductive cycle of rainbow trout can be advanced 12 wk (Bromage et al. 1982a; Whitehead et al. 1978). In female salmonids, oogonial proliferation and vitellogenesis are stimulated by long or increasing photoperiod while short or decreasing photoperiods regulate the final stages of oocyte maturation and ovulation (Whitehead et al. 1978; Whitehead and Bromage 1980; Bromage et al. 1982a; Bromage et al. 1984; Duston and Bromage 1987; Wallace et al. 1987). Furthermore, a gradual seasonal change in daylength is not necessary to stimulate reproductive development (Bromage et al. 1982b; Duston and Bromage 1986, 1988). Abrupt reductions in daylength can stimulate gonadal maturation and advance ovulatory responses (Whitehead et al. 1978; Duston and Bromage 1986; Duston and Bromage 1987). In this study, compressing a normal yearly cycle into 6 mo did not advance the reproductive cycle of Atlantic salmon females similarly to that of rainbow trout. However, the second 6-mo light cycle did induce spawning in all of the *2CP* females by the spring of the second year and reduced the time to rematuration by 6 mo.

The failure of salmon to respond to the first 6-mo photocycle may be associated with water temperature. Low water temperature may have blocked the initiation of exogenous vitellogenesis and prevented oocyte growth. Tyler et al. (1987) showed that the rate of vitellogenin uptake in rainbow trout follicles in vitro increased in response to increasing temperature. With a reduction in temperature there was a reduction in the rate of sequestering of vitellogenin by the oocyte. Also their study showed that vitellogenin uptake could occur at 5°C and that this temperature was within the physiological range for rainbow trout. Measurements of plasma estradiol levels for these females were very low during this period and indicated vitellogenesis was not proceeding.

The second 6-mo photocycle, however, did stimulate females into the vitellogenic phase by December and with the combined short photoperiod and 7°C water temperature between late December and late March egg development continued until final maturation in the spring. Although females were exposed to an increasing photoperiod from late March to June and a rising water temperature these environmental conditions did not arrest the development of the oocytes or their release. This observation is contrary to those recorded for rainbow trout (Duston and Bromage 1986, 1987, 1988). A similar observation was recorded in an ongoing study this year. In the present experiment water temperature during the winter was higher than seasonal conditions and this may have acted synergistically with the second 6-mo photocycle to bring about a greater hormone release and subsequent egg development. It is also possible that the first 6-mo photocycle may have removed any reproductive refractoriness and permitted the hypothalamo–pituitary–gonadal complex to respond to this greater hormone release. Regardless of the mechanism it is clear from these responses that if water temperature and body condition are above a certain threshold level and vitellogenesis has been stimulated oocyte development will proceed independently of photoperiod conditions. The data in the present study also support the findings of Tyler et al. (1987) and suggest that for Atlantic salmon once vitellogenesis is initiated, yolk deposition will continue at 6–7°C.

The response of the hypothalamo–pituitary–gonadal complex appears to be influenced also by the gain in body condition during the spring and early summer. Since salmon cease feeding upon entry into freshwater and may not feed again until their seaward migration the next spring, the development of oocytes requires mobilization and depletion of body lipids and proteins from liver and muscle storage sites. A large proportion of body mass must be mobilized for egg development. Failure to replace these body reserves by late summer appears to play an important role in controlling the reproductive process. This was evident in an earlier study (Johnston et al. 1987) and in this study for both males and females. The responsiveness of the hypothalamo–pituitary–gonadal complex may be determined by an interaction with growth or thyroid hormones. An analysis of thyroid hormone function is being undertaken to describe the seasonal profiles and role of T_3 and T_4 in gonadal maturation in these fish.

In males the condition factor for smaller grilse and larger reconditioned kelts indicated that a condition factor of 1.00–1.05 was necessary for spermatogenesis and spermiation. Although there was no difference in the rate of reconditioning of *2CP* and *NP* males there was a difference in the timing of spermiation. This difference can only be attributed to the heightened rate of testis development created by the first 6-mo photocycle. The hypothalamic–pituitary–gonadal complex in males appears to be more sensitive and responsive to light changes than females. The lower requirement for nutrient and energy mobilization from tissue compartments for spermatogenesis in males may be an important factor in determining the early responsiveness of males in this experiment. Males that failed to reach a critical level of energy storage through feeding remained refractory and without gonadal development.

In female *NP* spawners the increase in calcium followed by an increase in alkaline phosphatase levels suggested rising levels of vitellogenin in the plasma similar to earlier findings (Johnston et al. 1987). The increases in alkaline phosphatase activity and calcium levels are clearly related to increasing oocyte diameter, not oocyte number, and represent a useful managerial tool in salmonid culture. Nagler et al. (1987) showed a strong relationship between serum calcium and serum vitellogenin confirming that calcium can be used as an indirect indicator of vitellogenin levels. Alkaline phosphatase activity has been suggested as an important enzyme for creating a pool of phosphate for the hepatic synthesis of vitellogenin (Johnston et al. 1987). Calcium levels and alkaline phosphatase activity are clearly related to the process of vitellogenesis and related to each other. The alkaline phosphatase activity and calcium data presented here are useful in predicting the oocyte diameter and ovary weight. These parameters permit evaluation of broodstock potential, reproductive status, and oocyte size at considerably less cost than RIA assays of estrogens, androgens, or vitellogenin.

The increase in plasma cholesterol levels in females appears to be more closely related to weight gain and to a mobilization for reproductive function. The decline seen prior to spawning in *NP* spawners may be due to the synthesis of steroids and yolk components from cholesterol. The increase in concentration following spawning appeared to be a second mobilization perhaps due to high cortisol levels.

Triglycerides remained at basal levels until July with no apparent link to weight gain. While the triglyceride peak in *NP* spawners coincided with the calcium peak the roles of both cholesterol and triglyceride concentrations to oocyte development and reproductive function appear less clear than either alkaline phosphatase or calcium.

None of the blood chemistry parameters used in this study appear to be useful in evaluating or predicting the course of spermatogenesis or spermiation in males although plasma cholesterol and triglyceride levels appear to be linked to feeding and condition factor.

It is concluded from this study that a compressed seasonal light cycle did not improve either reconditioning or reproductive response of females in the first 12 mo, but it did effectively shift the spawning time of some males. A compressed seasonal photocycle, however, did induce spring spawning in females and reduced the time to rematuration in the second year by approximately 6 mo. Contrary to the findings of other studies, oocyte development and final maturation proceeded even though photoperiod was increasing and water temperature was low.

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Diel Migration and Microhabitat Distribution of a Benthic Stream Assemblage

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Rader, R. B., and J. V. Ward. 1990. Diel migration and microhabitat distribution of a benthic stream assemblage. Can. J. Fish. Aquat. Sci. 47: 711–718.

Macroinvertebrate microspatial distributions were determined on nonglazed brick substrates at three sites on the upper Colorado River. Site 1 was located above a reservoir, whereas Sites 2 and 3 were located 0.4 and 4.0 km, respectively, downstream from a deep-release dam. Replicate samples from each substrate surface (top, sides, front, back, and bottom) and from the interstices below brick substrates were collected during the day (ca 14:00–16:00) and at night (ca 21:00–23.00) in the summer and autumn of 1982. Although most macroinvertebrates occurred on "bottom" surfaces, "top," and "side" surfaces and the interstices were often utilized. Macroinvertebrates were least abundant on "back" and especially "front" surfaces. Total macroinvertebrate density on upper substrate surfaces was not significantly different at night compared with the day. However, there was a 47% increase in the density of *Baetis* spp. on upper surfaces during the night at Site 1, a diel shift in benthic substrate utilization that corresponded to drift periodicity. The density of *Baetis* spp. on upper surfaces during the night compared with the day was not significantly different at Sites 2 and 3. Large-sized *Baetis* spp. at Site 1, but not at Sites 2 and 3, used a greater proportion of upper substrate surfaces at night compared with the day.

Nous avons mesuré la microrépartition spatiale des macroinvertébrés sur des substrats de brique non vernie à trois sites du cours supérieur du Colorado. Le site 1 se trouve au-dessus d'un réservoir, tandis que les sites 2 et 3 sont situés respectivement à 0,4 et 4,0 km en aval d'un barrage à déversoir bas. Nous avons recueilli de façon répétée des échantillons sur chaque surface du substrat (dessus, côtés, devant, derrière et dessous) et dans les interstices au-dessous des substrats de brique pendant la journée (14h00 à 16h00 env.) et pendant la nuit (21h00 à 23h00 env.) au cours de l'été et de l'automne 1982. Si la plupart des macroinvertébrés se retrouvaient sur les surfaces du dessous, ils occupaient aussi souvent celles du dessus et des côtés, ainsi que les interstices. Les animaux étaient moins abondants sur les surfaces de derrière, et encore moins sur les surfaces de devant. La densité totale des macroinvertébrés sur les surfaces supérieures des substrats ne marquait pas de différence significative entre le jour et la nuit. On notait toutefois une augmentation de 47 % de la densité de *Baetis* spp. sur les surfaces du dessus pendant la nuit au site 1, modification nycthémérale de l'utilisation du substrat benthique qui correspondait à la périodicité de la dérive. La densité de *Baetis* spp. sur les surfaces du dessus la nuit par rapport au jour ne présentait pas de différence significative aux sites 2 et 3. Les *Baetis* spp. de grande taille, au site 1 mais non aux sites 2 et 3, utilisaient une plus grande proportion de la surface supérieure des substrats la nuit par rapport au jour.

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Several studies, both correlative and experimental, provide information concerning the factors which influence the microhabitat distribution of benthic macroinvertebrates in streams (e.g. Linduska 1942; Cummins 1964; Egglishaw 1969; Kohler 1983; Kovalak 1976; Rabeni and Minshall 1977; Hart 1978; Wise and Molles 1979; Reice 1980; Culp et al. 1983; Flecker and Allan 1984). These studies have examined patterns of macroinvertebrate distribution at a variety of spatial scales ranging from small plots to single stones. Very few studies, however, have used individual rock surfaces. Macroinvertebrates respond to environmental variables (e.g. current velocity and food patchiness) which vary over individual rock surfaces. The first objective of this study was to determine the microspatial distribution (substrate surface selection) of the macroinvertebrate assemblage at an unregulated, and a regulated and at a recovery site on the Upper Colorado River. We chose these sites in order to investigate the influence of stream regulation (flow constancy and high periphyton density) on the use of substrate surfaces by macroinvertebrates.

Diel changes in macroinvertebrate micro-habitat distributions can be related to explanations of diel drift periodicity (e.g. Allan et al. 1986). If periods of peak densities on upper substrate surfaces correspond to periods of peak drift then both active and passive mechanisms may explain the diel pattern of drift. However, if density on upper substrate surfaces and in the drift do not correspond, then active mechanisms must be operating. The second objective of this study was to determine the pattern of diel movements in the substrate and discuss its significance with respect to potential drift mechanisms.

Allan (1978 and 1984) found that a greater proportion of insects in larger size classes demonstrated a pattern of nocturnal drift. He suggested that large nymphs would tend to drift at night because of a greater mortality risk during the day due to

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size-selective predation by drift-feeding fish. In a nearly fishless stream, Casey (1987) found that small, not large, mayflies showed a diel shift in benthic substrate utilization by moving onto exposed surfaces at night. The third objective of this research was to determine if a greater proportion of large *Baetis* spp. nymphs utilized upper substrate surfaces during the night compared with daylight hours at three sites with abundant trout populations.

Methods

Site Descriptions

Three sampling stations (rubble riffle sites) were located in the upper Colorado River in the vicinity of Granby Reservoir in the state of Colorado, USA. Except for the influence of stream regulation, sites were similar with regard to elevation (2426–2593 m a.s.l.), gradient (0.006–0.009), canopy cover, geology, abundant trout populations, and riparian vegetation. The first site, an unregulated free-flowing riffle, was selected as a reference site. Site 2, 0.4 km downstream from Granby Dam, was located in an intensely regulated stream section. Site 3, located 4.0 km downstream from Granby Dam, exhibited at least partial recovery from the effects caused by the upstream impoundment. Complete site descriptions can be found in Rader and Ward (1988 and 1989).

Macroinvertebrate Micro-Habitat Distributions (Objective 1)

Microspatial distributions of the macroinvertebrate fauna were determined on dark brown, nonglazed, rectangular brick substrates (23 × 19 × 9.5 cm). Bricks with similar texture across all surfaces were chosen. Clay substrates are commonly used in benthic studies because they provide a uniform size and shape and because they are readily colonized by both periphyton and invertebrates (e.g. Kovalak 1979; Rosenberg and Resh 1982; Lamberti and Resh 1985; Tuchman and Stevenson 1980). Sixteen brick substrates were placed at 1-m intervals in four rows on the stream bed at each site with the long axis parallel to the flow. All brick substrates were placed in the stream 4 wk prior to sampling. Periphyton succession and accumulation on bare substrate requires approximately 3 wk (e.g. Korte and Blinn 1983; Reisen and Spencer 1970; Tuchman and Stevenson 1980). Brick surfaces were sampled for animals in June, July, September, and October of 1982 and June of 1983. Sampling of upper surfaces (top, sides, front, and back) consisted of sweeping (by hand) organisms into a net (30 × 20 cm rectangular opening; 240-μm mesh) positioned immediately downstream from the surface to be sampled. Eight different bricks were randomly chosen and used to sample four "top" surfaces and four "side" surfaces (one surface/brick). Four bricks were used to sample four "front" and four "back" surfaces. The "bottom" surface and "interstices" were sampled on four previously unsampled bricks. The "bottom" surface sample consisted of: (1) organisms swept from the bottom surface, and (2) organisms in the top centimetre of gravel, whereas the "interstices" sample consisted of organisms inhabiting the substrate from 1–10 cm below the brick. Before sampling the "bottom" and the "interstices," all organisms clinging to exposed surfaces were gently swept downstream. Then the brick was lifted and organisms on the "bottom" surface and for approximately 1 cm into the underlying gravel were swept into a Surber sampler (240-μm mesh). The substrate beneath the brick was agitated to approximately 10 cm and the dislodged organisms were swept into a second Surber sampler. All macroinvertebrates were preserved in 5% Formalin before sorting, identification, and enumeration. Microdistributional results based upon brushing macroinvertebrates from substrate surfaces could be biased for those species responsive to human movements. By quickly placing the net in position without disrupting the flow over the substrate, most macroinvertebrates on upper surfaces could be accurately sampled. For example, *Baetis* spp. nymphs were surprisingly docile in responding to human movements. However, data on *Cinygmula* spp., an extremely agile mayfly nymph (Allan et al. 1986), were excluded from this study because of its quick running response to human movements.

An analysis of variance was used to determine significant relationships between the dependent variable (macroinvertebrate density) and three main effects ("Position," "Site" and "Diel"). "Position" had six levels (top, sides, front, back, bottom, and interstices), whereas "Site" had three levels (Sites 1–3). "Diel" had two levels (day versus night samples). Macroinvertebrate density was calculated by dividing the area (cm^2) of each surface into the number of individuals collected per sample. Interstitial surface area was assumed to be 2× the bottom surface area. This assumption probably underestimated the amount of interstitial space and therefore, exaggerated the density estimates of the shallow interstitial habitat (<10 cm). Periphyton and detritus dry weight were included in the analysis as a covariate. The dry weights of periphyton (primarily filamentous green algae) and detritus (>240 μm) were determined from the same samples used to estimate macroinvertebrate densities on individual surfaces. Diagnostic procedures indicated that macroinvertebrate density and periphyton dry weight required a log transformation to stabilize the variance.

Diel Epibenthic Movements (Objective 2)

The prevalence of immigration and emigration in most stream communities (e.g. Townsend and Hildrew 1976) made it necessary to determine macroinvertebrate density on both the top and bottom of substrates during the day and at night. Four "top," "sides", and "bottom" surfaces and "interstices" were sampled at night between 21:00 and 23:00 at each site in July, September, and October (1982) on the same days that mid-afternoon (ca 14:00–16:00) samples were collected. Bricks used to sample "side" surfaces during the day were used at night to sample "top" surfaces, whereas bricks used to sample "top" surfaces during the day were used for "sides," and "bottom" surfaces and "interstices" at night. Sampling the same brick but different surfaces during the day and again at night did not appear to affect the night samples because mobile organisms (e.g. *Baetis* spp.) had adequate time to recover and slow-moving and sessile organisms were unaffected. A battery-operated head lamp covered with red cellophane was used to locate brick substrates at night. Macroinvertebrates were collected in nets, as opposed to direct observations, because visual surveillance (especially at night) was difficult on the "top" and "side" surfaces. However, drift from upstream sources could have biased our results if drifting nymphs represented a substantial portion of the total number of nymphs collected. Since sampling events were of short duration (<30 s), this concern seemed unwarranted.

An analysis of variance was used to determine if there were significant relationships between the dependent variable and three main effects ("Surface," "Site," and "Diel"). Total macroinvertebrate density and *Baetis* spp. density were used as

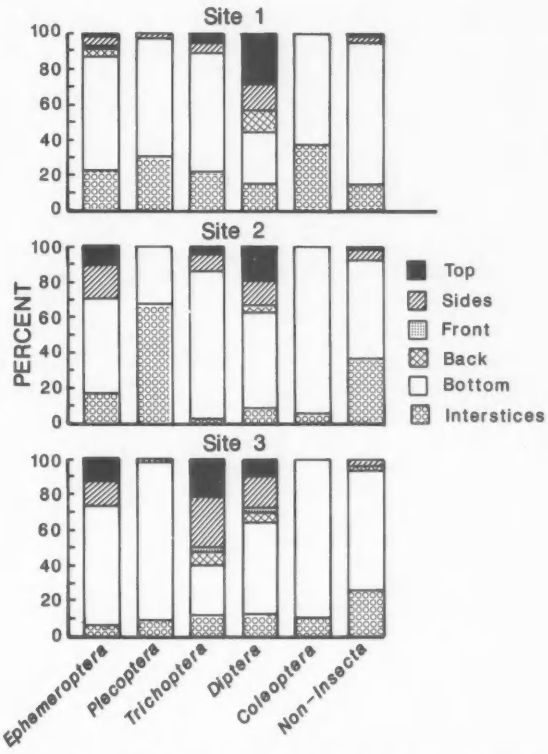

FIG. 1. Percent of macroinvertebrate density by taxonomic orders utilizing brick substrate surfaces and the interstices at each site. "Non-Insecta" included Turbellaria, Nematoda, Oligochaeta, *Hydra* spp. and Hydracarina.

response variables in separate analyses. "Surface," "Site," and "Diel" were the same as above.

Baetis Body Size/Diel Relationship (Objective 3)

Baetis spp. nymphs were collected from each surface during the day and night as described above. All nymphs were dried at 60°C for 48 h and placed in one of four size classes (0.01–0.25, 0.251–0.5, 0.51–0.75, and >0.751 mg). An analysis of variance was used to determine if there were significant relationships between the response variable (*Baetis* spp. density) and three main effects ("Position," "Size," and "Diel"). "Position" had two levels, "exposed" (combined samples from top and side surfaces) and "concealed" (combined samples from bottom and interstices), whereas "size" had four levels corresponding to the four size classes. "Diel" was the same as described above.

Results and Discussion

Macroinvertebrate Micro-Habitat Distributions (Objective 1)

As expected, macroinvertebrates did not utilize all surfaces equally; "Position" of invertebrates on substrate surfaces was a significant ($P<0.001$) main affect. Table 1 summarizes the density data for selected taxa at each site. Although most macroinvertebrates occurred on the "bottom" surface, both "top" and "side" surfaces and the interstices were often utilized (Fig. 1). Only an occasional chironomid or the tricopteran *Glossosoma* spp. occurred on "front" surfaces which were oriented perpendicular to the force of the flow (Table 1). Nor were macroinvertebrates abundant on the "back" surfaces where flow tends to spiral in turbulent vortices (Osborne et al. 1985). The Diptera, Trichoptera, and Ephemeroptera (minus *Baetis* spp. and *Cinygmula* spp.) utilized a greater proportion of exposed surfaces ("top," "sides," and "front") compared with the Plecoptera, Coleoptera, and non-Insecta, which utilized primarily "bottom" surfaces and interstices (Fig. 1).

Macroinvertebrate micro-habitat distribution differed significantly ($P<0.001$) between sites and the abundance of macroinvertebrates on substrate surfaces was significantly ($P<0.001$) correlated ($r^2 = 0.96$) with the dry weight biomass of periphyton and detritus. Species eliminations and reductions and habitat modifications (dense growths of filamentous algae) resulting from stream regulation (see Rader and Ward 1988) account for the site differences. Periphyton density on upper substrate surfaces and detritus accumulations were strongly correlated with macroinvertebrate density because both are important determinants of macroinvertebrate microdistributions (e.g. Minshall 1984). None of the interactions between the main effects from the analysis was significant.

Except for *Baetis* spp. which will be considered separately, *Pseudocloeon* spp. and *Siphlonurus occidentalis* (Eaton) were the primary Ephemeroptera utilizing exposed surfaces at Site 1 (Table 1). However, thick mats of filamentous green algae at Sites 2 and 3 allowed a greater percentage of typically cryptic mayflies (*Ephemerella infrequens*, McDunnough; *Drunella grandis*, Eaton; *Paraleptophlebia heteronea*, McDunnough) to occupy exposed surfaces. Dorso-ventrally flattened heptageniid mayflies (*Epeorus longimanus*, Eaton; *Epeorus albertae*, McDunnough and *Rhithrogena hageni*, Eaton) primarily utilized "bottom" surfaces at each site. Although the Plecoptera at Site 2 preferred the interstitial habitat (Fig. 1), only two taxa (*Amphinemura banksi*, Baumann and Gaufin; *Isoperla* spp.) were collected. The much more diverse plecopteran fauna at Sites 1 and 3 (Rader and Ward 1988) preferred "bottom" surfaces (Table 1). *Glossosoma* spp. were rare at Site 2 but accounted for most of the Trichoptera occupying exposed surfaces at Sites 1 and 3. At Site 2, *Rhyacophila* spp. found in the filamentous algae were the only Trichoptera using exposed surfaces. The most abundant Trichoptera (*Arctopsyche grandis*, McLachlan and *Rhyacophila* spp. at Site 1, *Hesperophylax designatus*, Banks and *Oligophlebodes minutus*, Ulmer, at Site 2 and *Lepidostoma ormeum*, Rambur and *Glossosoma* spp. at Site 3) primarily inhabited "bottom" surfaces and/or the interstices (Table 1). Chironomids and simuliids were the dominant dipterans on exposed surfaces at each site, whereas a variety of taxa utilized "bottom" surfaces and the interstices (e.g. *Hexatoma* spp., *Wiedemania* spp., *Bezzia* spp., *Atherix* spp. and numerous chironomid species). The Coleoptera exclusively used concealed surfaces (Fig. 1). A greater standing crop of sedimentary detritus at Site 1 compared with both sites downstream from the dam (Rader and Ward 1988) may account for the larger percentage of Coleoptera using the interstitial habitat at Site 1. At all three sites, *Hydra* spp. were the major non-Insecta inhabiting "top" and "side" surfaces, whereas the turbellarian *Polycelis coronata* (Girard), Hydracarina, and

TABLE 1. The mean densities (numbers/cm^2) of selected taxa are shown for each substrate surface during the day and night at each site. Values in parentheses represent the standard deviation for each mean. Surfaces 1, 2, 3, 4, 5, and 6 represent top, sides, front, back, bottom, and interstices, respectively.

Taxa	Site	Day/Night	Surface 1	2	3	4	5	6
Baetis spp	1	D	0.0060 (0.0112)	0.0320 (0.0187)	0.0277 (0.0055)	0.1069 (0.0681)	0.0954 (0.0360)	0.0028 (0.0026)
		N	0.0292 (0.0137)	0.1075 (0.0466)	— —	0.0055 (0.0060)	0.0453 (0.0024)	0.0015 (0.0021)
	2	D	0.1077 (0.0260)	0.2823 (0.1107)	0.0792 (0.0603)	0.1301 (0.1551)	0.4661 (0.1663)	0.0401 (0.0632)
		N	0.2741 (0.1038)	0.9121 (0.0567)	0.0664 0.0254)	0.1457 (0.1096)	0.7929 (0.0503)	0.0463 (0.0186)
	3	D	0.1077 (0.0028)	0.2347 (0.0979)	0.0216 (0.0254)	0.1052 (0.1157)	0.2032 (0.0803)	0.0173 (0.0148)
		N	0.2144 (0.1665)	0.3981 (0.2178)	0.0193 (0.0188)	0.0554 (0.0415)	0.2258 (0.1029)	0.0140 (0.0120)
Epeorus longimanus	1	D	—	—	—	0.0265 (0.0155)	0.0167 (0.0066)	—
		N	—	—	—	—	—	—
	2	D	—	—	—	—	—	—
		N	—	—	—	—	—	—
	3	D	0.0029 (0.0013)	0.0045 (0.0045)	— —	0.0094 (0.0033)	0.0938 (0.0045)	— —
		N	—	0.0105 (0.0082)	— —	— —	0.0823 (0.0457)	— —
Ephemerella infrequens	1	D	0.0006 (0.0011)	0.0045 (0.0036)	— —	0.0072 (0.0033)	0.3313 (0.1678)	0.0110 (0.0036)
		N	—	—	—	—	0.0677 (0.0258)	0.0048 (0.0024)
	2	D	0.0281 (0.0171)	0.0265 (0.0137)	— —	0.0027 (0.0033)	0.0784 (0.0254)	0.0042 (0.0073)
		N	0.0091 (0.0130)	0.0860 (0.0379)	— —	— —	0.0572 (0.0032)	0.0185 (0.0096)
	3	D	0.0366 (0.0315)	0.1281 (0.0718)	— —	0.0470 (0.0653)	0.0915 (0.0379)	0.0110 (0.0129)
		N	0.0876 (0.0773)	0.0961 (0.1432)	— —	0.0055 (0.0011)	0.1434 (0.0320)	0.0125 (0.0016)
Drunella grandis	1	D	—	—	—	—	0.0018 (0.0009)	0.0003 (0.0005)
		N	—	—	—	—	0.0016 (0.0011)	—
	2	D	0.0048 (0.0038)	0.0169 (0.0082)	—	—	0.0075 (0.0027)	—
		N	—	0.0100 (0.0137)	—	—	—	—
	3	D	0.0057 (0.0059)	0.0173 (0.0100)	—	0.0083 (0.0072)	0.0114 (0.0082)	0.0026 (0.0017)
		N	0.0098 (0.0102)	0.0183 (0.0064)	—	—	0.0235 (0.0199)	0.0014 (0.0026)
Paraleptophlebia heteronea	1	D	—	—	—	—	0.0496 (0.0327)	0.0057 (0.0029)
		N	—	—	—	—	0.0528 (0.0409)	—
	2	D	0.0084 (0.0050)	0.0192 (0.0082)	—	—	0.00075 (0.0011)	—
		N	0.0018 (0.0020)	0.0164 (0.0096)	—	—	0.0070 (0.0043)	—
	3	D	—	—	—	—	—	—
		N	—	—	—	—	—	—
Siphlonurus occidentalis	1	D	0.0001 (0.0025)	0.0402 (0.024)	—	—	0.0267 (0.0192)	0.0033 (0.0019)
		N	0.0022 (0.0027	0.0091 (0.0022)	—	—	0.0308 (0.0279)	—
	2	D	—	—	—	—	—	—
		N	—	—	—	—	—	—
	3	D	—	0.0128 (0.0100)	—	—	0.0842 (0.0443)	0.0048 (0.0033)
		N	—	0.0059 (0.0096)	—	—	0.1304 (0.0354)	0.0030 (0.0052)

TABLE 1. (*continued*)

Taxa	Site	Day/Night	Surface 1	2	3	4	5	6
Amphiremura banksi	1	D	—	—	—	—	0.0016 (0.0025)	—
		N	—	—	—	—	0.0020 (0.0041)	—
	2	D	—	—	—	—	0.0016) (0.0013)	0.0003 (0.0005)
		N	—	—	—	—	—	—
	3	D	—	—	—	—	0.0059 (0.0043)	0.0021 (0.0010)
		N	—	—	—	—	0.0073 (0.0045)	0.0009 (0.0013)
Taenionema spp.	1	D	—	0.0013 (0.0022)	—	—	0.0132 (0.0102)	0.0014 (0.0005)
		N	0.0040 (0.0011)	0.0059 (0.0059)	—	—	0.0068 (0.0137)	0.0003 (0.0005)
	2	D	—	—	—	—	0.057 (0.0020)	—
		N	—	—	—	—	—	—
	3	D	—	—	—	—	—	—
Glossosoma spp.	1	D	0.0064 (0.0022)	0.0045 (0.0036)	0.0016 (0.0033)	0.0016 (0.0044)	0.0077 (0.0041)	0.0013 (0.0006)
		N	—	0.0013 (0.0022)	—	—	0.00098 (0.0050)	—
	2	D	—	—	—	—	—	—
		N	—	—	—	—	—	—
	3	D	0.1233 (0.1070)	0.3011 (0.2778)	0.0188 (0.0182)	0.0947 (0.1019)	0.0125 (0.0082)	0.0014 (0.0017)
		N	0.0922 (0.0784)	0.2897 (0.1070)	—	0.0808 (0.0858)	0.3105 (0.1528)	0.0251 (0.0279)
Lepidostoma ormeum	1	D	—	—	—	—	—	—
		N	—	—	—	—	—	—
	2	D	—	—	—	—	—	—
		N	—	—	—	—	—	—
	3	D	0.0004 (0.009)	0.0137 (0.0157)	—	—	0.0739 (0.0324)	0.0035 (0.0026)
		N	0.0006 (0.0013)	0.0045 (0.0064)	—	—	0.0221 (0.0098)	—
Rhyacophila spp.	1	D	—	—	—	—	0.0118 (0.0025)	0.0014 (0.0009)
		N	—	—	—	—	0.0086 (0.0048)	—
	2	D	0.0038 (0.0048)	0.0032 (0.0027)	—	—	0.0082 (0.0027)	—
		N	—	0.0050 (0.0086)	—	—	0.0089 (0.0050)	—
	3	D	—	—	—	—	0.0096 (0.0070)	0.0025 (0.0012)
	N	—	—	—	—	—	0.0130 (0.0144)	—
Polycelis coronata	1	D	—	—	—	—	0.0052 (0.0027)	0.0016 (0.0012)
		N	—	—	—	—	0.0089 (0.0045)	0.0010 (0.0008)
	2	D	—	—	—	—	0.0018 (0.0006)	0.0011 (0.0009)
		N	—	—	—	—	0.0070 (0.0061)	—
	3	D	—	0.0068 (0.0086)	—	0.0072 (0.0066)	0.02006 (0.1498)	0.0163 (0.0141)
		N	0.0040 (0.0048)	0.0022 (0.0045)	—	—	0.1709 (0.0274)	0.0072 (0.0056)

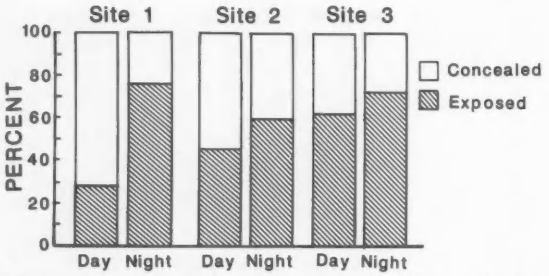

FIG. 2. Percent of *Baetis* spp., based on density estimates, utilizing exposed ("top" and "side" surfaces) versus concealed ("bottom" surface and interstices) micro-habitat space during the day and at night.

Nematoda primarily comprised the non-Insecta on "bottom" surfaces and in the interstices (Table 1).

Diel Epibenthic Movements (Objective 2)

The relationship between drift periodicity and benthic diel migration remains unclear. In some investigations, increased densities on the upper surfaces of stones at night appeared to correspond with periods of peak drift (Elliott 1968; Campbell 1980; Bailey 1981; Casey 1987; Wilzbach et al. 1988), whereas other studies demonstrated either greater densities during the day (Graesser and Lake 1984; Statzner and Mogel 1985; Allan et al. 1986) or no difference between day and night density estimates (Clifford 1972; Kovalak 1979; Kohler 1983). With respect to the total macroinvertebrate assemblage, this study demonstrated that there were no significant ($P = 0.87$) density differences on the upper surfaces of stones at night compared with the day. Similarly, the "Diel" main effect was not significant ($P = 0.21$) when data for *Baetis* spp., a common member of the drift, were analyzed separately. However, the "Diel" × "Site" interaction term ($P = 0.07$) suggested that there were significant differences in the density of *Baetis* spp. on exposed surfaces during the night compared with the day at one or more sites. At Site 1, there was a 47% increase in the density of *Baetis* spp. on exposed surfaces at night compared with the day (Fig. 2 and Table 1). Also, a majority of *Baetis* spp. occurred on concealed surfaces during the day and exposed surfaces at night. The density of *Baetis* spp. on exposed surfaces at Site 1 did not correspond to periods of peak drift; therefore, both active and passive mechanisms could explain drift periodicity at this site. Although *Baetis* spp. utilized a greater proportion of exposed surfaces at night compared with the day at Sites 2 and 3, this trend was not significant (Fig. 2). The abundance of filamentous algae downstream from the dam increased habitat heterogeneity and probably diminished prey detection by fish, providing refugia from predators and the current, on upper substrate surfaces making them more similar to concealed bottom surfaces. Therefore, *Baetis* spp. at Sites 2 and 3 may utilize upper and lower surfaces with equal frequency without experiencing increased predation on current-related dislodgement which are typically associated with upper, exposed surfaces.

A review of the literature indicated no relationship between the methods used to detect a response and the diel movement of mayflies from concealed to exposed surfaces. Half of the six studies demonstrating an increase in density on upper substrate surfaces during the night used a substrate sampling technique (Elliott 1968; Campbell 1980, Site 1 of this study), whereas, the other half used observational methods (Bailey 1981 and 1982; Wilzbach et al. 1988; Casey 1987). One out of three studies demonstrating a decrease in density on exposed surfaces during the night used a substrate-sampling technique (Graesser and Lake 1984). The remaining two used observational methods (Statzner and Mogel 1985; Allan et al. 1986). Three out of four studies indicating no difference in density between exposed and concealed surfaces from day to night used a substrate sampling technique (Clifford 1972; Kovalak 1979; Sites 2 and 3 of this study), while, one used observational methods (Kohler 1983). It appears that response differences may be attributable to natural biological variation and not necessarily methodological biases.

Substrate sampling techniques, compared with observational methods, provide information on the distribution of organisms across all substrate surfaces and are equally accurate during the day or at night. Viewing box observational methods do not disturb the substrate but can only record data from upper surfaces and often require a bright red light for night observations (Casey 1987). Although some mayflies responded similarly during the daylight hours to artificial white light, red light, and no artificial light (Casey 1987), aquatic insects may be able to detect and avoid red light (Harker 1953), especially bright sources of red light at night. The nighttime response of aquatic insects to various intensities of red light should be tested against observations using infrared light. Night observations, however, have been accomplished using dim sources of red light (e.g. Allan et al. 1986) and ambient light intensifiers (Statzner and Mogel 1985). Poor visual acuity may be a problem with both methods, but especially when using light intensifiers. Most night observations can be best accomplished using video cameras (e.g. Peckarsky and Wilcox 1989) with infrared capabilities. Recent technology (fiber optics with infrared enhancement) has allowed relatively undisturbed observations over small portions of the bottom surface where interstitial openings are sufficiently large (Wilzbach et al. 1988). At present, fiber optics has been used primarily in laboratory experiments (Wilzbach et al. 1988). Each method has advantages and disadvantages which must be considered with respect to site-specific environmental conditions, and the hypothesis being tested.

Baetis Body Size/Diel Relationship (Objective 3)

Smaller sized *Baetis* spp. (0.01–0.1 mg) at Site 1 utilized a greater proportion of exposed surfaces (day and night) compared with larger *Baetis* spp. (Fig. 3). This trend, however, was not observed at either Sites 2 or 3. The large-sized *Baetis* spp. (0.45–>1.0 mg) at site 1 also utilized a significantly ($P < 0.05$) greater proportion of exposed surfaces during the night compared with the day (Fig. 3). This pattern was not evident for large-sized *Baetis* spp. at Sites 2 and 3. The growth of abundant filamentous algae at both sites below the dam likely provided cover for *Baetis* spp. utilizing exposed surfaces. Therefore, the risk of predation from visual predators was reduced and nymphs did not use exposed surfaces more frequently during the night compared with the day. Therefore, only data from Site 1 supports the hypothesis that the density of large nymphs on exposed surfaces should increase at night to reduce predation from visually feeding predators (i.e. trout). Although Casey's (1987) data did not support this hypothesis, he was working in a small second order stream with "no fish presently in the study area." All three sites in this study support abundant populations of (*Oncorhynchus mykiss*) (Richardson), *Salmo trutta* (Linnaeus) and *Salvelinus fontinalis* (Mitchell). It appears, therefore, that

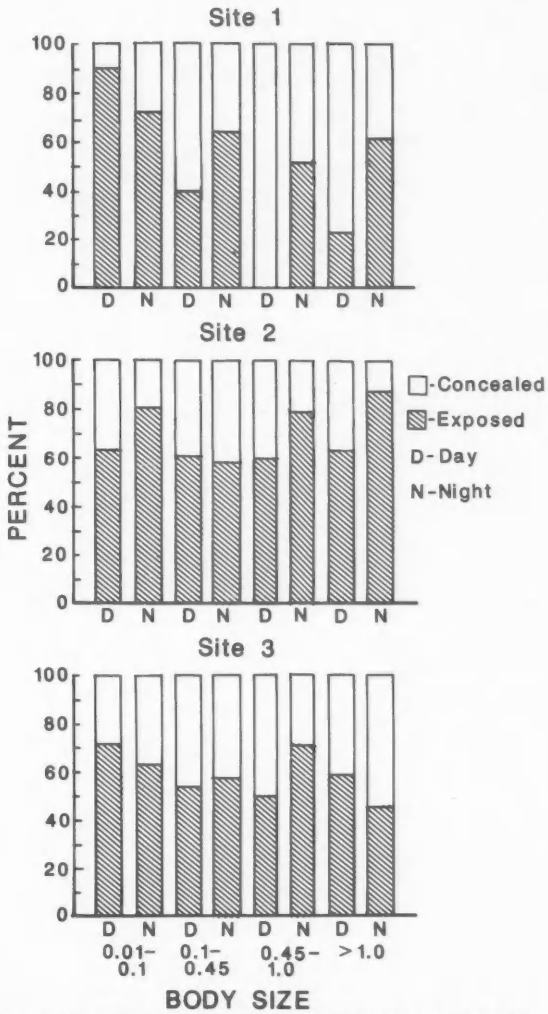

FIG. 3. Percent density of four *Baetis* spp. body size categories utilizing exposed ("top" and "side" surfaces) versus concealed ("bottom" surface and interstices) micro-habitat space during the day and at night. Size categories represent milligrams of dry weight biomass.

where the risk of predation is high (e.g. Site 1 where upper substrate surfaces are not covered with filamentous algae and moss), larger nymphs may become increasingly more nocturnal in their use of exposed substrate surfaces.

Summary and Conclusions

Stream regulation indirectly influenced macroinvertebrate microhabitat utilization in the upper Colorado River. Constant flow conditions (daily and seasonally) reduce the influence of scouring and increases bed substrate stability allowing abundant growths of periphyton to cover upper substrate surfaces, thereby providing prey refugia and altering microcurrent flow conditions across exposed substrate surfaces. Several of the larger sized mayfly species (e.g. *Drunella grandis*) as well as other typically cryptic macroinvertebrate taxa were able to use upper substrate surfaces because of the periphyton cover. Although the total macroinvertebrate fauna did not shift from concealed to exposed substrate surfaces on a diel basis, *Baetis* spp. density at Site 1 on exposed surfaces significantly increased at night due to the behavior of the larger size classes. This pattern corresponds with periods of peak drift densities and is therefore consistent with both passive and active explanations for drift periodicity in streams. Furthermore, the movement of large bodied *Baetis* spp. onto exposed substrate surfaces during the night at Site 1 supports previous discussions concerning fish predation and the proportionately greater number of large-sized *Baetis* spp. in the drift (Allan 1978). Periphyton growth on upper substrate surfaces at sites downstream from the dam provided predator and current refugia allowing *Baetis* spp. to utilize bottom and upper surfaces with roughly equal frequency during the day and night.

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Crossing Methodology and Genotypic Diversity in a Hatchery Strain of Rainbow Trout (*Oncorhynchus mykiss*)

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Gile, S. R., and M. M. Ferguson. 1990. Crossing methodology and genotypic diversity in a hatchery strain of rainbow trout (*Oncorhynchus mykiss*). Can. J. Fish. Aquat. Sci. 47: 719–724.

Genotype frequencies and heterozygosity at nine polymorphic enzyme loci were determined in 720 rainbow trout (*Oncorhynchus mykiss*) produced by three types of crosses: full-sib family, diallel, and pooled gamete. Five pairs of parental fish were crossed by each method within each of three experiments. Offspring produced by pooled crosses deviated significantly from equal parental genetic contributions while those from the diallels did not. Significant deviation in full-sib family offspring is attributable to only a single locus in one experiment. The deviations in the pooled cross may be the result of unequal male contributions. Cross types also differed significantly in genotype frequencies. Crossing methodology has potentially important effects on the genetic variation of cultured fishes.

Les fréquences des génotypes et l'hétérozygosité dans neuf sites enzymatiques polymorphes ont été déterminées dans 720 truites arc-en-ciel (*Oncorhynchus mykiss*) produites par trois types de croisements : fratrie, croisement diallèle et gamètes en pool. Cinq couples de poissons parents ont été croisés selon chacune des méthodes dans chacune des trois expériences. Les petits produits par croisement en pool déviaient considérablement par rapport aux contributions génétiques parentales égales, ce qui n'était pas le cas des croisements diallèles. Un écart important chez les petits des croisements de fratries est attribuable à un seul lieu dans une expérience. Les écarts dans les croisements en pool pourraient être les résultats d'une contribution inégale des mâles. Les types de croisement différaient également considérablement dans la fréquence des génotypes. Les méthodes de croisement ont pu avoir des effets importants sur la variation génétique des poissons élevés.

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Fish culture plays an important role in the conservation and rehabilitation of exploited or endangered populations of fishes. Conservation programs should have the preservation of genetic variation as a primary goal (Frankel and Soulé 1981; Meffe 1986; Allendorf and Ryman 1987; Nelson and Soulé 1987). Genetic variation as estimated by enzyme heterozygosity has been correlated with components of fitness (Mitton and Grant 1984; Allendorf and Leary 1986; Zouros and Foltz 1987). Low enzyme heterozygosity has been associated with deleterious effects in hatchery fishes (Leary et al. 1985). Population size is one of the most important factors influencing the amount of genetic variation and stability of genotypic frequencies in captive populations, as large effective population sizes minimize deleterious genetic effects.

Hatchery spawning procedures can affect the amount of genetic variation in fish produced, if they affect the relative contribution of individual parents. Two types of crosses are commonly used in fish culture, single pair and pooled gamete matings. Single pair matings ensure equal parental contributions at fertilization whereas pooled matings do not. For example, male salmon do not contribute equally in pooled matings, thus reducing the effective population size (Gharrett and Shirley 1985; Withler 1988).

We examined the effects of typical crossing methodology used in fish culture on the genotypic diversity of progeny. We tested whether or not the genotype frequencies of rainbow trout (*Oncorhynchus mykiss*) produced by three crossing methods (full-sib family, full diallel, pooled gamete) deviate from parental expectations assuming that all parents contribute equally. We also determined whether or not the crosses differed in genotype frequencies and heterozygosity.

Materials and Methods

Three-year-old rainbow trout (*Oncorhynchus mykiss*) were collected from the Ontario Ministry of Natural Resources Normandale Fish Culture Station on March 15, 1988. These individuals were progeny of an original brood stock established by collection of gametes from several hundred wild fish captured in the Ganaraska River at Port Hope, Ontario during the early 1980's. Gametes were collected in dry plastic containers and packed on wet ice for transport to the University of Guelph. Gametes were kept at 4°C until the experimental matings were carried out within 12 h of collection. Samples of eye, muscle, and liver were removed from parental fish and frozen in liquid nitrogen.

Fifteen males and 15 females were subdivided at random into three experiments, each with five pairs of fish. Three types of crosses were produced with gametes from the 10 parents per experiment. Randomly pairing single males and females resulted in five full-sib families. Diallel crosses were produced by crossing each male with each female resulting in 25 progeny groups. In the pooled gamete mating, equal volumes of eggs from each of the five females were mixed. Likewise, equal volumes of sperm from each of the five males were first mixed and then combined with the eggs.

TABLE 1. Observed allele frequencies in five pairs of parental rainbow trout crossed by three methods in three experiments.

		Experiment 1		Experiment 2		Experiment 3	
Locus	Allele	Males	Females	Males	Females	Males	Females
Aco2	100	0.9	1.0	1.0	1.0	1.0	1.0
	84	0.1	0	0	0	0	0
G3p1	100	1.0	1.0	0.9	0.9	0.9	0.9
	140	0	0	0.1	0.1	0.1	0.1
Hex	100	1.0	0.9	1.0	0.9	0.9	0.7
	75	0	0.1	0	0.1	0.1	0.3
Idh2	100	0.7	0.7	0.8	1.0	0.9	0.7
	140	0.3	0.3	0.2	0	0.1	0.3
Idh3,4[a]	100	0.95	0.80	0.85	0.75	0.70	0.90
	114	0	0.10	0.05	0	0.05	0.05
	71	0	0.05	0	0.10	0.05	0.05
	40	0.05	0.05	0.10	0.15	0.20	0
Mdh3,4[a]	100	0.70	0.70	0.80	0.90	0.95	0.70
	125	0.10	0.05	0	0.05	0	0.05
	83	0.15	0.05	0.20	0	0	0.25
	74	0.05	0.20	0	0.05	0.05	0
Me3	100	1.0	1.0	1.0	1.0	0.9	1.0
	90	0	0	0	0	0.1	0
Pgm2	100	1.0	1.0	0.9	0.8	0.7	0.6
	90	0	0	0.1	0.2	0.2	0.3
	120	0	0	0	0	0.1	0.1
Sod	100	0.7	0.7	0.5	0.6	0.8	0.8
	152	0.2	0.3	0.4	0.4	0.2	0.2
	50	0.1	0	0.1	0	0	0

[a]Frequencies at the duplicated loci, *Mdh3,4* and *Idh3,4* were determined with allele counting.

The progeny groups were placed in separate sections of vertical incubation trays. After hatching, equal numbers of offspring from each of the five full-sib families within an experiment were combined into a single group. Similarly, equal numbers of progeny from each of the 25 diallel groups were combined. The pooled gamete cross remained unaltered. Thus, each experiment was composed of three treatment groups. The fish were reared for 5 mo in flowing well water ranging from 7 to 13°C and fed commercial trout food. Numbers of mortalities were recorded throughout the rearing period. The offspring were killed with tricane methanesulfonate (MS222) or 2-phenoxyethanol and frozen at −80°C.

Enzymes produced by the following polymorphic loci were examined in parental fish and progeny by starch gel electrophoresis, following the methods of Allendorf et al. (1977): aconitate hydratase (*Aco2*; EC 4.2.1.3), glycerol-3-phosphate dehydrogenase (*G3p1*; EC 1.1.1.8), hexosaminadase (*Hex*; EC 3.2.1.30), isocitrate dehydrogenase (*Idh2; Idh3,4*; EC 1.1.1.42), malate dehydrogenase (*Mdh3,4*; EC 1.1.1.37), malic enzyme (*Me3*; EC 1.1.1.40), phosphoglucomutase (*Pgm2*; EC 5.4.2.2) and superoxide dismutase (*Sod*; EC 1.15.1.1). Eighty progeny were examined from each cross type in each experiment ($N = 720$).

Results

Parents and Offspring

We first determined with *G*-tests (goodness of fit) if offspring genotype frequencies deviated from parental expectations assuming that all parents contributed equally. In the pools and diallels, expected frequencies in progeny were obtained by multiplying the frequencies of alleles in the male and female parents (Table 1). This assumes that all males fertilize all females and therefore is invalid for the full-sib families. The expected frequencies in full-sibs were calculated within each family according to Mendelian expectations and then summed over the five families within an experiment. Since the *G*-statistic is additive (Sokal and Rohlf 1981), it could be summed for a cross type across all loci within an experiment and across all experiments. The duplicated loci (*Mdh3,4* and *Idh3,4*) were not included in this analysis, as it is difficult to predict expected offspring genotype frequencies due to residual tetrasomic segregation during meiosis (Allendorf and Thorgaard 1984).

The three cross types showed differential congruence with parental expectations (Table 2). Pooled gamete progeny showed significant deviations from parental expectations in two experiments. The *G*-statistic summed over all experiments indicated a highly significant deviation from parental expectations. Full-sib families showed significant deviations in one of three experiments and the combined statistic over all experiments. However when individual loci were considered, only one (*Sod* in Experiment 1) deviated significantly and accounted for by far the largest proportion of the combined statistic. *Idh2* genotypes in experiment 3 did not deviate significantly if the total number of tests is taken into account (critical $P = 0.05/6 = 0.0083$) (Cooper 1968). Diallel cross progeny did not deviate significantly from expectations in any single experiment or across all three.

Among Cross Types

Progeny genotype frequencies differed significantly among cross types in all three experiments (contingency *G*-test) (Table 3). Further analysis indicated that the genotype frequencies of diallels versus pools differed significantly (Total $G = 48.76$, $df = 28$, $P = 0.008$), as did pools and diallels combined versus full-sibs (Total $G = 100.57$, $df = 29$, $P < 0.001$).

The mean number of heterozygous loci per fish (standard error) for the full-sibs, pools, and diallels was 2.40 (0.09), 2.60 (0.09), and 2.52 (0.08), respectively. These data were analysed with a two-way ANOVA with experiment, cross, and interaction as sources of variation. There were no significant differences among cross types (experiment: $F_{[2,711]} = 30.317$, $P < 0.001$; cross: $F_{[2,711]} = 1.508$, $P = 0.222$; interaction: $F_{[4,711]} = 2.467$, $P = 0.044$).

Mortality

There was significant heterogeneity in the survival of progeny produced by the different crosses (contingency *G*-test) (Table 4). This variation was not, however, consistently associated with any one cross type. For instance, the full-sibs showed the lowest mortality in experiment 1 whereas in experiment 2 these fish had the greatest mortality from hatching to sampling. Mortality appeared to be stochastic with regard to cross type and experiment.

Discussion

The genetic goals of fish culture will differ depending upon the intended use of the fish produced (Allendorf and Ryman 1987). For instance, in aquaculture where rapid growth rates and food conversion efficiency may be desirable characteristics, selective breeding is often used to alter the genetic com-

TABLE 2. Comparison of rainbow trout offspring genotype frequencies observed in three types of crosses in three experiments versus those expected with equal parental contributions (most genotypic classes with <5 expected were combined with the next largest class).

	Cross					
	Full-sib family		Diallel		Pool	
Locus	*G* (df)	*P*	*G* (df)	*P*	*G* (df)	*P*
Experiment 1						
Aco2	1.13 (1)	0.287	0.00 (1)	1.000	17.04 (1)	<0.001
Hex	0.00 (1)	1.000	0.13 (1)	0.718	0.00 (1)	1.000
Idh2	1.14 (2)	0.565	6.30 (2)	0.043	16.19 (2)	<0.001
Sod	20.06 (2)	<0.001	5.74 (3)	0.125	6.48 (3)	0.090
Total	22.33 (6)	0.001	12.17 (7)	0.095	39.71 (7)	<0.001
Experiment 2						
G3p1	0.30 (1)	0.583	5.58 (1)	0.018	0.41 (1)	0.523
Hex	1.96(1)	0.162	0.14 (1)	0.708	0.52 (1)	0.471
Idh2	0.08 (1)	0.777	0.08 (1)	0.777	0.08 (1)	0.777
Pgm2	3.31 (2)	0.191	1.86 (1)	0.173	9.02 (2)	0.011
Sod	2.28 (3)	0.516	3.13 (3)	0.372	3.13 (3)	0.372
Total	7.93 (8)	0.444	10.79 (7)	0.148	13.16 (8)	0.106
Experiment 3						
G3p1	0.08 (1)	0.777	5.58 (1)	0.018	0.41 (1)	0.520
Hex	3.46 (1)	0.062	1.16 (1)	0.281	1.73 (1)	0.188
Idh2	8.48 (2)	0.014	1.16 (1)	0.281	2.86 (1)	0.091
Me3	0.00 (1)	1.000	0.13 (1)	0.718	2.67 (1)	0.102
Pgm2	1.45 (3)	0.694	1.22 (4)	0.874	9.16 (4)	0.057
Sod	2.51 (1)	0.113	2.73 (1)	0.098	2.03 (1)	0.154
Total	15.98 (9)	0.067	11.98 (9)	0.214	18.86 (9)	0.026
Grand total	46.24 (23)	0.003	34.94 (23)	0.053	71.73 (24)	<0.001

position of the cultured population. In conservation programs, however, two objectives are to minimize changes in allele frequencies from one generation to the next and maintain genetic variation.

Our results show that crossing methodology affects the stability of genotype frequencies across generations. The genotypes of offspring produced by pooling the gametes from several males and females showed the greatest deviations from parental expectations. This observation agrees with a previous study of rainbow trout (Danzmann and Ferguson 1988). One explanation for these deviations is that all parents, specifically males, do not contribute equally in pooled crosses. Two studies with genetically marked Pacific salmon males have shown clearly that such differential contributions of males can occur (Gharrett and Shirley 1985; Withler 1988).

Deviations in the pooled crosses could also be explained by the differential survival of offspring with specific genotypes. In other words, genotype frequencies of offspring at fertilization may conform to parental expectations but differential mortality would result in the observed deviations in 5-mo-old fish. Our evidence suggests that this is not the case. Both the pools and diallels would be expected to have the same set of genotypes if all males fertilize all females equally. Thus, any deleterious genotypes should be present in both groups, leading to parallel deviations. The lack of deviations in the diallels refutes this hypothesis. Moreover, the differential mortality hypothesis predicts that greater mortality should occur in the cross with the greater deviations. We have no evidence for increased mortality in the pooled gamete crosses relative to the diallels. Finally, the lack of consistency between cross types and experiments in the specific loci that deviated from parental expectations suggests that selection against particular genotypes is an unlikely explanation for our results.

Differential fertilization success of males is not a tenable explanation for any deviations from parental expectations detected in the full-sib progeny because single males and females were crossed, thereby precluding any sperm competition. In addition, identical numbers of fish were combined from each family after hatching, thus equalizing contributions. Differential survival of families after they were combined, could contribute to deviations in the progeny sampled for electrophoresis. We had no method of counting family specific mortality after the families were combined. Our data do show that differential family specific mortality occurred when fish were still in the incubating trays. If we assume that the relative mortality among families is constant over different ontogenetic periods, differential mortality of families is a potential contributing factor to deviations. However, since deviations observed for all loci were insignificant except for one case, the full-sib family offspring essentially do not deviate from equal parental genetic contributions. The large deviation of *Sod* genotypes is not consistent across experiments and is likely to be of minimal biological significance.

In addition to the differential deviations from parental expectations among the crosses, significant differences in genotype frequencies were detected between crosses. The greatest variability occurred between the full-sibs and the other two cross types. This is not surprising given that different genotypic combinations are expected due to the mechanics of the crosses. Such differences would be reduced if greater numbers of parents were used, as is typical of most hatcheries. These data do suggest, however, that cross type has the potential to affect

TABLE 3. Observed numbers of rainbow trout with different genotypes produced by three crossing methods in three experiments.

Locus	Genotype	Cross			G^a (df)	P
		Full-Sib	Diallel	Pool		
Experiment 1						
Aco2	100/100	69	72	59	8.09 (2)	0.018
	100/90	11	8	21		
Hex	100/100	72	71	72	0.09 (2)	0.957
	100/75	8	9	8		
Idh2	100/100	38	50	56	12.78 (4)	0.012
	140/100	31	26	22		
	140/140	11	4	2		
Idh3,4	100/100	49	56	63	5.90 (2)	0.052
	Het.[b]	31	24	17		
Mdh3,4	100/100	17	18	15	0.36 (2)	0.837
	Het.[b]	63	62	65		
Sod	100/100	51	48	38	11.83 (4)	0.019
	152/100	28	24	33		
	152/152	1	5	2		
	100/50	0	2	7		
	152/50	0	1	0		
Total					39.05 (16)	0.001
Number Het. Loci (SE)		2.15 (.11)	1.91 (.15)	2.15 (.12)		
Experiment 2						
G3p1	100/100	62	56	67	4.32 (2)	0.115
	140/100	18	24	13		
	140/140	0	0	0		
Hex	100/100	68	73	70	1.53 (2)	0.466
	100/75	12	7	10		
Idh2	100/100	63	65	64	0.16 (2)	0.925
	140/100	17	15	16		
Idh3,4	100/100	26	17	29	4.79 (2)	0.091
	Het.[b]	54	63	51		
Mdh3,4	100/100	48	36	44	3.76 (2)	0.153
	Het.[b]	32	44	36		
Pgm2	100/100	61	52	45	14.87 (4)	0.005
	100/90	13	27	28		
	90/90	6	1	7		
Sod	100/100	25	23	20	15.73 (4)	0.003
	152/100	22	40	43		
	152/152	26	13	11		
	100/50	7	4	3		
	152/50	0	0	3		
Total					45.16 (18)	<0.001
Number Het. Loci (SE)		2.18 (.13)	2.80 (.15)	2.49 (.15)		
Experiment 3						
G3p1	100/100	63	56	67	4.14 (2)	0.110
	140/100	17	23	12		
	140/140	0	1	1		
Hex	100/100	56	55	56	0.04 (2)	0.981
	100/75	24	22	19		
	75/75	0	3	5		
Idh2	100/100	42	55	43	5.42 (2)	0.064
	140/100	36	23	36		
	140/140	2	2	1		
Idh3,4	100/100	34	23	35	4.69 (2)	0.096
	Het.[b]	46	57	45		
Mdh3,4	100/100	41	37	32	2.05 (2)	0.359
	Het.[b]	39	43	48		
Me3	100/100	72	71	76	2.37 (2)	0.305
	100/90	8	9	4		

TABLE 3. *(Continued)*

Locus	Genotype	Cross: Full-Sib	Cross: Diallel	Cross: Pool	G^a (df)	P
Pgm2	100/100	44	35	34	20.25 (6)	0.002
	100/90	16	26	27		
	120/100	5	12	16		
	90/90	9	3	2		
	120/90	2	4	1		
	120/120	4	0	0		
Sod	100/100	41	44	45	16.31 (4)	0.001
	152/100	39	29	26		
	152/152	0	7	9		
Total					55.27 (22)	<0.001
Number Het. Loci (SE)		2.89 (.18)	3.10 (.13)	2.94 (.15)		

[a]Most genotypic classes with <5 expected were combined with the next largest class.
[b]All heterozygous classes were combined.

TABLE 4. Mortality of rainbow trout produced by three types of crosses in three experiments.

Experiment		Cross: Full-sib	Cross: Diallel	Cross: Pool	G (df)	P
To hatching[a]						
1	Alive	898	3948	815	117.99 (2)	<0.001
	Dead	92	1121	134		
	Percent mortality	9.3	22.1	14.1		
2	Alive	824	3757	839	89.03 (2)	<0.001
	Dead	161	774	52		
	Percent mortality	16.3	17.1	5.8		
3	Alive	652	4160	835	151.75 (2)	<0.001
	Dead	414	1507	205		
	Percent mortality	38.8	26.6	19.7		
Hatching to sampling at 5 mo						
1	Alive	394	884	601	12.36 (2)	0.002
	Dead	106	366	214		
	Percent mortality	21.2	29.3	26.3		
2	Alive	355	1051	705	9.14 (2)	0.010
	Dead	100	199	134		
	Percent mortality	22.0	15.9	16.0		
3	Alive	202	1010	717	26.28 (2)	<0.001
	Dead	78	240	118		
	Percent mortality	27.9	19.2	14.1		

[a]Mortalities to hatching include any unfertilized eggs, the proportions of which are assumed to be equal among crosses which used the same parental fish.

genotype frequencies in cultured fishes. In fish culture programs aimed at supplementing natural populations, careful consideration must be given to crossing methodology, so that equal parental representation and effective population size are maximised.

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Virus-Like Particles in an Ultra-Oligotrophic Lake on Vancouver Island, British Columbia[1]

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Transmission electron microscopy (TEM) seasonal studies of concentrated water samples from Sproat Lake, Vancouver Island, British Columbia, revealed numerous polygonal virus-like particles of variable size (60–200 nm). These particles (ca. 10^7/mL) were either free-living or associated with host picoplankters. Negative staining of living samples provides clear evidence of early stages of phage–picoplankton interactions. These phages display a six-sided head (ca. 90 nm dia.) with a distinct appendage (ca. 200 nm) or striated tail (ca. 130 nm). Viruses with dense matrices, deprived of envelopes or occurring as empty shells were found in the marginal area of invaded cells. Morphological changes such as invagination of the photosynthetic lamellae with the appearance of 'virogenic stroma' or with disruption of the cell membrane and the cell wall are described. Comments on the possible functional significance of viral agents in the biology and ecology of host cells are presented.

Des études saisonnières par microscopie électronique par transmission (TEM) d'échantillons d'eau concentrée prélevés dans le lac Sproat dans l'île Vancouver en Colombie-Britannique ont révélé la présence de nombreuses particules viroïdes polygonales de taille variable (60–200 nm). Ces particules (environ 10^7/mL) vivaient de façon autonome ou associée à du picoplancton hôte. La coloration négative d'échantillons vivants a fait ressortir clairement l'existence de stades hâtifs d'interaction bactériophage–picoplancton. Ces bactériophages ont une tête hexagonale (environ 90 nm de diamètre) et un appendice distinct (environ 200nm) ou une queue striée (environ 130 nm). Des virus à matrices denses, privés d'enveloppes ou se présentant sous forme de carapaces vides, ont été trouvés dans la zone marginale des cellules envahies. Des changements morphologiques tels que l'invagination des lamelles photosynthétiques avec apparition de « stroma virogène » ou rupture de la membrane cellulaire et de la paroi cellulaire sont décrits. Des commentaires sur l'importance fonctionnelle possible des agents viraux dans la biologie et l'écologie des cellules hôtes sont présentés.

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Virus-like particles have long been recognized as a ubiquitous component of aquatic ecosystems, either free living or associated with various procaryotic and eucaryotic organisms, e.g. bacteria, cyanobacteria, and algae (Brown 1972; Padan and Shilo 1973; Pienaar 1975; Melnick 1978; Mayor and Taylor 1979; Johnson and Sieburth 1982; Dodds 1983; Moebus 1983; Reisser et al. 1986; Sieburth et al. 1988; Bergh et al. 1989). Some of these studies have focused on the morphology and physico–chemical characteristics of viruses while others have dealt with host response and viral–host interactions. The absence of suitable methods to allow rapid field detection and reliable enumeration of virus-like particles are factors that are largely responsible for our present poor understanding of their ecology in the pelagic of both freshwater and marine ecosystems. There is a paucity of information on the seasonal distribution and patterns of abundance of both free and host-associated viruses (see Bergh et al. 1989; Proctor and Fuhrman 1990).

Algal blooms are a common occurrence in most lacustrine and coastal marine systems, but very little is known about the role viruses may play in their control or collapse (Safferman and Morris 1964; Safferman et al. 1972; Dodds 1983). This report describes for the first time associations of virus-like particles with picoplankton and other protists in an ultra-oligotrophic lake and discusses their possible effects on cyanobacterial populations.

Description of Study Lake

Sproat Lake is located in the south-central region of Vancouver Island (49°14′N, 125°06′W), an area with warm, dry summers and cool, wet winters. The lake is warm-monomictic, with a mean depth of approximately 59 m and a theoretical water residence time of 8 yr. Littoral areas are steep-sided and narrow, and the lake is divided into four distinct basins, separated by shallow sills. The surrounding topography and orientation of the lake act to shield it from prevailing winds, with the result that there is relatively little wind-induced vertical mixing with a stable summer thermocline (ca. 10 m). The drainage basin of Sproat Lake has shallow, sandy soils underlain by impervious igneous rock which result in generally low dissolved ion concentrations and low biological productivity (Stockner 1987). The lake is an important nursery area for sockeye salmon (*Oncorhynchus nerka*), which spend 1–2 yr in the lake prior to seaward migration.

Methods

Bimonthly samples were taken from a single station in Sproat Lake from the surface (1 m) from October 1987 to September

[1]This paper is dedicated to Dr. Thana Bisalputra on the occasion of his retirement from a prominent career in cell biology at the University of British Columbia, Vancouver, B.C.

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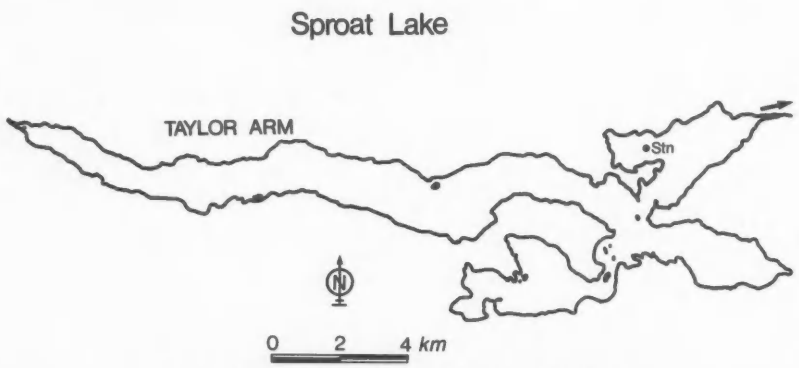


FIG. 1. Sproat Lake showing location of sampling station.

1988 (Fig. 1). Samples were kept cool (ca. 4°C) until processed, usually within 24 h of collection. Millipore 0.22-μm polymer filters were placed in acid-cleaned and double-distilled water rinsed Millipore or Gelman filtration units and between 20 and 300 mL of sample were filtered at a vacuum pressure differential of between 50–90 mm Hg (680–1225 Pa).

Filtered samples were fixed at room temperature for 2 h with 2% glutaraldehyde containing 0.05% of Alcian Blue in 0.1 M sodium cacodylate buffer (pH 7.5), and postfixed for 1 h in 1% osmium tetroxide with 0.05% Ruthenium Red using the same buffer. After embedding in 1% agar, cell samples were dehydrated with a graded ethanol series and embedded in Epon 812. Ultrathin sections were double-stained with uranyl acetate and lead citrate and viewed with a Zeiss EM 10A electron microscope. For negative staining, freshly obtained samples were centrifuged, mounted on formvar-coated grids and stained for 5 min. with 1.5% uranyl acetate.

To estimate virus-like particle abundance, 50 transmission electron micrographs were randomly chosen and a virus/bacteria (V/B) ratio computed from counts made of bacteria and viruses present on each micrograph. The acridine orange direct count (AODC) method with epiflorescence microscopy was used to enumerate free-living bacteria in Sproat Lake from 1984–86 (Hobbie et al. 1977; Shortreed and Stockner 1990).

Results

Transmission electron microscopy (TEM) seasonal studies of concentrated water samples from Sproat Lake revealed numerous virus-like particles that were notably more abundant in the spring than at other seasons of the year. These particles were retained by adsorption to the surface of the filters and were either suspended in the water (Figs. 2–5), attached to the cell surface coat of eucaryotic picoplankters (Fig. 6), or found inside bacteria and unicellular cyanobacteria (Figs. 7–9).

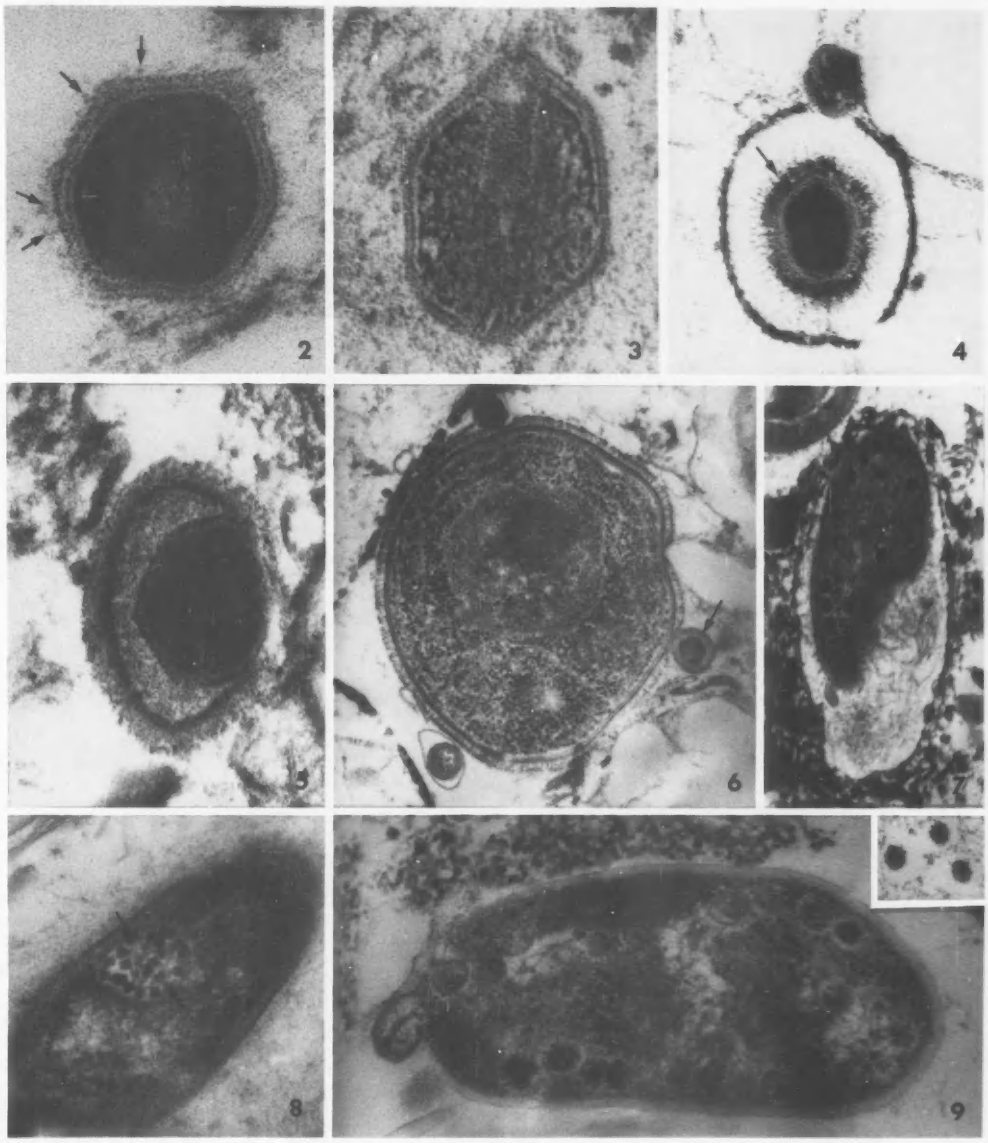
Bacteria numbers in Sproat Lake ranged from lows of 6×10^4/mL in winter to highs of 1 to 1.5×10^6/mL in the epilimnion in late summer (Shortreed and Stockner 1990). Examination of 50 electron micrographs revealed the following: (1) most viruses occurred as 'free-living' particles, (2) their average density was 10/section (each section was 10 nm thick), and (3) their ratio of abundance relative to bacteria (V/B) was 3:1.

Virus-like particles were polyhedron shaped, with a five to six sided head capsid, and ranged in diameter from 60–200 nm. Each of these units was encased in a triple layered shell (Figs. 2 and 3). This envelope contained capsomeres (arrows, Fig. 2), or a continuous opaque-layer about 60 nm thick (arrow, Figs. 4 and 6), reminiscent of a glycoproteinaceous coat known from most living cells. Interestingly, some of these virus-like particles were confined to a membrane-bound compartment possibly derived from the host upon emergence (Fig. 5).

When seen free from host cells, virus-like particles showed two types of internal structure: (1) a less dense, finely granular core surrounded by what is presumed to be genetic material compactly coiled in various (ca. six) concentric layers (Fig. 2), and (2) a more heterogeneous appearance, containing an unravelled cylindric tail (130 nm long, 40 nm wide) attached to one of the vertices via the capital head (described here as a latent state of a phage) (Fig. 3).

Among the infected picoplankters, some showed signs of substructural changes such as numerous arrays of ribosomes and displaced photosynthetic lamellae with clear areas between the cell wall and the thylakoid (virogenic stroma), containing aggregates of virus precursors (e.g. arrows, Fig. 8). In other host cells (e.g. *Synechococcus*), mature viruses with dense cores and empty shells were often separated by electron transparent halos (also called zones of exclusion), usually found in the marginal area of the cytoplasm (Figs. 7 and 9). In one picoplanktonic host, it was possible to recognize intermediate stages of virus replication and assembly (Fig. 9). Some of these stages do not reveal envelopes and they appear to be released into the aquatic environment by disruption of the cell wall (Fig. 9, inset). It was also found that some infected eucaryotic picoplankton cells (ca. 1.8 μm) displayed a great number of viruses (>30) as well as remains of organelles.

Additional ultrastructural observations of centrifuged living samples negatively stained with uranyl acetate provided evidence of some early stages of the phage invasion process. These phages (80–100 nm dia.) were found to interact with the cell surface of apparently phototrophic picoplankters (0.8–1.5 μm length), via a thin appendage (ca. 200 nm in length, Figs. 10 and 11) or by the virus head (Figs. 12 and 13). Interestingly, in this last case (Fig. 13), a striated tail with a distal end exhibits the same length (130 nm) as that recorded for the unfolded tail of an apparent 'latent' phage. It was also noted that some of these target picoplanktonic cells collected in the early spring (April) may have just divided (Fig. 12), suggesting that the physiological state of susceptible cells may play an important role in their vulnerability to virus infection.

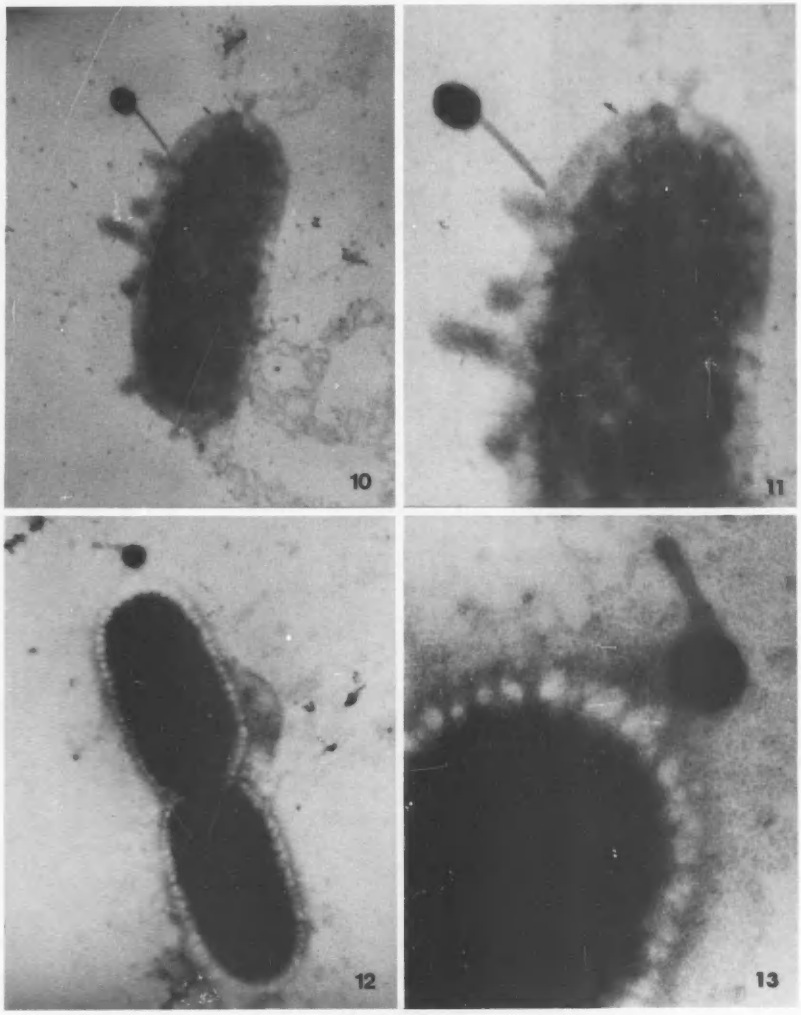


Figures 2–9.

FIG. 2. Virus-like particle with six sides. Note the genetic material coiled and the capsomeres (arrows) of the triple-layered shell (× 221 540); FIG. 3. 'Latent' stage of a phage with a retracted tail (× 222 920); FIG. 4. Five-sided virus-like particle depicting an anionic surface layer stained with Alcian Blue–Ruthenium Red (arrow) (× 100 800); FIG. 5. Virus enclosed in a membrane-bound compartment (× 143 530); FIG. 6. Two virus-like particles attached to the surface coat of a eucaryotic cell. The arrow indicates the electron-dense coat of a virus particle (× 34 040); FIG. 7. *Synechococcus* infected by viruses. Note the invaginated thylakoids and the halo around the particles (× 30 560); FIG. 8. Cyanobacterium depicting a 'virogenic stroma' (arrows) (× 21 680); FIG. 9. Picoplankter with virus at different stages of replication (× 58 400). Inset, naked virus-like particles found in the vicinity of infected cells. (× 30 560).

Discussion

Seasonal ultrastructural studies of a natural algal community from Sproat Lake revealed abundant polygonal virus-like particles, ranging in size from 60–200 nm, occurring both in a free-living (unattached) state or associated with algal cells. Although in nature host-range specificity is difficult to assess, size variability among viral agents may give some clues about the nature and diversity of their hosts. Viruses with similar morphological characteristics have been previously identified in lymphocytes of fish (Weissenberg 1965), erythrocytes of reptiles, insect larvae (Pickett-Heaps 1972), African swine (Hess 1971), protozoa, and some procaryotic and eucaryotic algae (Padan and Shilo 1973; Mayor and Taylor 1979; Dodds 1983;



Figures 10–13

FIG. 10, 11. Phage infecting a cyanobacterium. Note the six-sided head and the long appendage (× 47 630; × 91 350); FIG. 12, 13. Phage attacking an apparently dividing cyanobacterium. Note the striated tail and the head adhered to the glycocalyx of the cyanobacterium (× 49 500, × 166 670).

Sieburth et al. 1988). The Sproat Lake viruses were often found infecting the most common cyanobacterial picoplanker in the lake — *Synechococcus*. Viruses (phages) attacking much larger colonial cyanobacteria were first isolated from a waste stabilization pond (Safferman and Morris 1963), and currently they are known to have a widespread geographical distribution (Padan and Shilo 1967; Safferman and Morris 1967; Singh and Singh 1967; Daft et al. 1970; Pienaar 1976).

Although direct quantitative enumeration of viruses can be difficult (see Moebus 1983), by using the 3:1 V/B ratio we were at least able to obtain an estimate of their abundance, albeit conservative. Since the average number of bacteria in the epilimnion of the lake is between 5–7 × 10^5/mL (Shortreed and Stockner 1990), a minimal estimate of virus-like particle abundance would be 1.5–2 × 10^6/mL. With their extremely small size range (60–200 nm), one would expect the majority to pass an 0.2-μm nominal pore diameter filter (Brock 1983; Stockner et al. 1990). If the 0.2-μm filter retains only 10% of free, virus-like particles then our estimate of the viral population would be >10^7/mL, which is not unlike values recently reported by Bergh et al. (1989) from a variety of aquatic ecosystems. It should be noted that both our estimate and those reported by Bergh et al. (1989) place virus-like particles, or *femtoplankton* (Sieburth et al. 1978), as the most abundant and ubiquitous particles in the pelagial of both marine and freshwater ecosystems.

It is known that some viruses can tolerate extreme environmental conditions (Padan and Shilo 1973), including chlorination for the treatment of drinking water (Payment 1981), and they can survive for periods of up to 6 mo (dormancy) without a host cell (Melnick 1978). During dormancy or periods of inactivation we observed that some phages kept their tails retracted,

and it is possible that the captial head may play an important role in the switch to the infectious state upon encountering favorable conditions.

It has been reported that the rate of viral infection is not only regulated by environmental variables such as light, temperature, pH, and nutrients, but also by the inherent properties of the susceptible host cells, e.g. presence or absence of specific receptor sites, genome and the metabolic state of the target cell (Brown 1972; Padan and Shilo 1973). Interestingly, virus infection of Sproat Lake cyanobacterial picoplankton, e.g. *Synechococcus*, was observed mainly in the early spring (March–April). Previous studies have shown that at this time of the year the lake has sufficient nutrients to sustain algal division and growth (Suttle and Harrison 1988; Shortreed and Stockner 1990). Such high rates of metabolic activities are believed to encourage the infection of target cells by opportunistic viral agents that depend on the host cell for replication. During this process, the nucleic acid of the virus (possibly DNA) reaches the nucleoplasm of the host, and hybrids, mutant or resistant forms may occur naturally as a result of the confluence between genomes. It has recently been pointed out that high viral abundance in natural waters may well contribute to the high turnover rates of bacteria as well as to their mortality and great diversity as a result of active genetic recombination (Sherr 1989; Proctor and Fuhrman 1990).

The substructural changes we have seen in infected picoplankton cells (e.g. ribosomes, cell wall, and thylakoids) are similar to those reported for the colonial cyanobacteria *Anabaena variabilis* and *Plectonema boryanum*, where they have been attributed to biochemical and physiological changes (Smith et al. 1966; Granhall and Hofsten 1969; Padan and Shilo 1973). Although some investigators believe that the displacement of the thylakoids reflects alterations in photosynthesis, others have opined that their collapse results from the breakdown of nucleic acids (Padan and Shilo 1973). In contrast to these structural changes and similarity to *Synechococcus* invaded by SR-1 (MacKenzie and Haselkorn 1972), some infected cyanobacteria in Sproat Lake did not show major morphological alterations. It is possible that during host–virus interaction, an equilibrium is established for much of the host life cycle. During such periods of mutual tolerance, the invader undergoes a state of latency which seems to be important in the survival of infected algae (Dodds 1983). In some algal species the ultimate stage of infection — lysogeny, only occurs after zoospore formation. Lysogeny of algal cells is commonly observed in natural aquatic habitats, and can be induced naturally, via autolysis, or through the action of bacteria or viral agents. Recent studies on virus infected *Chlorella* have shown that a cell wall degrading enzyme (lysin) is responsible for the rupture of the host cell (Schuster et al. 1987). Interestingly, viral lytic activity has long been regarded as an important tool to arrest blooms of potentially harmful colonial cyanobacteria (Safferman and Morris 1963, 1964; Shilo 1967).

With our observations of high viral densities and their infectious effects, has come the possibility that they may play an important role in algal succession, and the seasonal wax and wane of phototrophic biomass and production. Changes in algal communities have been attributed to a number of variables, including predation, nutrient depletion, light, accumulation of metabolites, parasitism, and pH (Shapiro 1973; Lange 1974; Vincent and Silvester 1979; Reynolds 1984). Although it is difficult to assess the extent to which viruses may influence the dynamics of a natural population, their lytic action may be an important factor controlling the abundance of microalgae and influencing nutrient recycling in pelagic communities. It was noteworthy that cyanobacteria, although seen infected, were never dominant in the spring when free phages were abundant. Because cyanobacterial picoplankton and bacteria are such important components of pelagic communities (Stockner and Antia 1986; Stockner and Porter 1988), further studies are now urgently required to investigate the ecological role and implications of such viral agents in the control of picoplankton abundance and diversity.

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Thermal Tolerances of the Predatory Cladocerans *Bythotrephes cederstroemi* and *Leptodora kindti*: Relationship to Seasonal Abundance in Western Lake Erie

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Abundances of two predatory cladocerans, recently introduced *Bythotrephes cederstroemi* (Schoedler) and native *Leptodora kindti* Focke, in western Lake Erie were negatively correlated in 1987, with the invader abundant in late autumn and the native more abundant in summer. Laboratory experiments were conducted in summer and autumn of 1988 to determine if differences in thermal tolerance could explain species replacement in these two ecologically similar species. Body mass, sex ratio, fecundity, and population abundance were also measured in summer and autumn populations. Mortality of *Bythotrephes* increased with temperature, while mortality of *Leptodora* increased both above and below ambient lake temperatures. *Leptodora* was less tolerant of low temperature (5–15°C), and both species were equally tolerant of high temperature (20–30°C). Seasonal acclimation to temperature was absent in *Bythotrephes*, while *Leptodora* showed seasonal shifts in thermal tolerance. Reduced fecundity, greater percentage of males, and presence of sexual eggs were evidence that *Bythotrephes* reached an environmental limit during summer. Decreased abundance of *Leptodora* in autumn can be explained by sensitivity to declining water temperature, but warm water temperature alone cannot explain numerical dominance of *Leptodora* in summer.

L'abondance de deux cladocères prédateurs, *Bythotrephes cederstroemi* (Schoedler), récemment introduit, et *Leptodora kindti* Focke, une espèce autochtone, dans l'ouest du lac Érié a été corréliée de façon négative en 1987; l'envahisseur abonde à la fin de l'automne et l'espèce autochtone est plus abondante en été. Des expériences en laboratoire ont été effectuées en été et en automne 1988 afin de déterminer si les différences dans la tolérance thermique pouvaient expliquer le remplacement de ces deux espèces présentant des caractéristiques écologiques similaires. La masse corporelle, la proportion des sexes, la fécondité et l'abondance de la population ont également été mesurées dans les populations estivales et automnales. La mortalité de *Bythotrephes* augmentait avec la température tandis que celle de *Leptodora* augmentait autant à des températures supérieure qu'à des températures inférieures à la température ambiante du lac. *Leptodora* tolérait moins les basses températures (5–15°C) et les deux espèces toléraient également les températures élevées (20–30°C). *Bythotrephes* ne présentait aucune acclimatation saisonnière à la température, tandis que *Leptodora* affichait des changements saisonniers dans sa tolérance thermique. Une réduction de la fécondité, une augmentation du pourcentage des mâles et la présence d'oeufs témoignent de l'atteinte d'une limite environnementale pour *Bythotrephes* en été. L'abondance moindre de *Leptodora* en automne peut s'expliquer par sa vulnérabilité à la baisse des températures de l'eau, bien que la chaleur de l'eau seule ne peut expliquer sa dominance numérique en été.

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Seasonal change of water temperature is an important factor regulating population dynamics of freshwater organisms. In Cladocera, environmental temperature determines short-term metabolic functions, including overall activity, metabolic rate, embryonic development rate, growth rate, and size at first reproduction, and long-term responses, such as triggering formation of males and sexual resting eggs within the population (Brown 1929; Pratt 1943; Johnson 1952; Schindler 1968; Armitage and Lei 1979; Culver 1980). Populations of cladocerans are physiologically and genetically adapted to local seasonal temperature patterns. Interspecies and intraspecies (=clonal) variation in thermal tolerance and acclimation are correlated with peak summer water temperature in *Daphnia* (Johnson 1952; MacIsaac et al. 1985; Carvalho 1987). However, little attention has been paid to variation in thermal tolerance of trophically similar species, or the role temperature plays in species succession in zooplankton communities (MacIsaac et al. 1985).

Bythotrephes cederstroemi (Schoedler), a predatory cladoceran native to oligotrophic lakes of northern Europe, has recently invaded the North American Great Lakes (Bur et al. 1986; Lange and Cap 1986; Lehman 1987; Cullis and Johnson 1988). This exotic species is ecologically similar to a native Great Lakes predatory cladoceran, *Leptodora kindti* Focke, and the ranges of these two species overlap in Europe. Both species prey on smaller cladocerans, copepods, and rotifers by external grinding followed by sucking prey fluids into the mouth (Mordukhai-Boltovskaia 1956, 1958, 1960; Monakov 1972).

Patterns of reproduction are also similar in both species. Parthenogenetic females dominate the population until late in the year when males are produced, followed by sexual resting eggs (overwintering stage) (Mordukhai-Boltovskaia 1957; Andrew and Herzig 1984). Environmental temperature and thermal history are likely inducers of sexual stages of both species (Andrew and Herzig 1984; Herzig 1985).

Patterns of dispersal of *Bythotrephes* through the North American Great Lakes point to a single introduction into Lake Huron, probably via freighter ballast water (Bur et al. 1986). Subsequent to initial discovery in Lake Huron in 1984, *Bythotrephes* was recorded from Lakes Erie and Ontario in 1985, Lake Michigan in 1986, and Lake Superior in 1987 (Bur et al. 1986; Lange and Cap 1986; Lehman 1987; Cullis and Johnson 1988). Populations founded from a single introduction, representing perhaps only a few viable individuals, often have greatly reduced intrapopulation genetic variability (founder effect). North American *Bythotrephes* populations have little or no genetic variability as revealed by starch gel electrophoresis at 12 enzyme loci (Garton and Berg 1990; D. J. Berg and D. W. Garton, unpubl. data). Recent studies have shown positive relationships between individual genetic variability, phenotypic plasticity, and fitness in many invertebrate species (reviewed by Mitton and Grant 1984; Zouros and Foltz 1987). Therefore, this lack of genetic variation in recently established populations of *Bythotrephes* may result in reduced ability to acclimate fully to seasonal environmental changes in its new habitat.

The ecological impact of *Bythotrephes* on trophic dynamics of zooplankton communities within the Great Lakes has also attracted attention (Lehman 1987; Berg and Garton 1988; Evans 1988; Garton and Berg 1990). An initial study in 1987 on seasonal abundance of *Bythotrephes* and *Leptodora* in western Lake Erie revealed significant negative correlation between these two predatory cladocerans (Berg and Garton 1988). When *Leptodora* was present, *Bythotrephes* was absent; rarely were both species present in the same sample. In addition, abundance of *Leptodora* was positively correlated and *Bythotrephes* negatively correlated with lake temperature, and peak abundance of *Bythotrephes* was transient, preliminary evidence that the invader was poorly adapted to seasonal thermal regimes in the western basin of Lake Erie (Berg and Garton 1988). This suggests that environmental temperature was responsible for seasonal abundance of these two species in western Lake Erie in 1987. Accordingly, we measured thermal tolerance of summer and autumn populations of these two species to determine whether species-specific thermal tolerances could explain observed seasonal species replacement, and to determine if seasonal thermal acclimation of *Bythotrephes* was less complete than *Leptodora*. Abundance, demographic profiles, fecundity, and standardized dry weights were also determined for summer and autumn populations.

Methods and Materials

Laboratory experiments were conducted to compare within-species differences in thermal tolerance of summer versus autumn populations of *Bythotrephes* and *Leptodora*. The experimental design also allowed comparison of between-species differences in thermal tolerance.

Cladocerans were collected by vertically hauling a plankton net at a site near Put-in-Bay, OH, in the western basin of Lake Erie. Individual animals of both species were placed in 20 × 150 mm culture tubes filled with filtered lake water and held at ambient lake temperature for 18–24 h. The acclimation period allowed time for animals injured during collection to die, leaving uninjured animals for determining thermal tolerance. Animals were not fed during acclimation or experimental periods, a total of 30–36 h. Culture tubes containing live animals were transferred directly to constant temperature water baths (10, 15, 20, 25, 30, and 35°C, summer experiment, and 5, 10, 15, 20, 25, and 30°C, autumn experiment). Water baths were covered by styrofoam lids during the experiment. Water bath temperature and cladoceran mortality were measured hourly for 12 h. Animals were considered dead when all movement ceased. Twelve-hour LT_{50} values were calculated using the PROBIT procedure in the Statistical Analysis System (SAS) (SAS, Inc. 1985). Seasonal differences in the shape of temperature-mortality curves were tested using a three-way $R \times C$ G-test for interaction (Sokal and Rohlf 1969). Between-species (within season) and between-season (within species) differences in percent mortality were tested using an equality of percentages method (Sokal and Rohlf 1969) at each experimental temperature.

Summer experiments (10–35°C) were conducted 18–21 July 1988, when Lake Erie water temperature was 25°C. Sample size (number of animals per temperature treatment) ranged from 31–34, except at 25°C, which was replicated (sample size 66–69). Autumn experiments (5–25°C) were conducted 21–22 October 1988 (lake temperature 10.5°C) for *Leptodora* and 5–6 November 1988 for *Bythotrephes* (lake temperature 7.1°C). Because population abundances were low, the 30°C-treatment was not conducted until 11–12 November 1988 (lake temperature 6.8°C) for both species. Sample size ranged from 23–39 animals per treatment.

Lake temperature and seasonal abundance of *Bythotrephes* and *Leptodora* were monitored by sampling every 7–10 d from May through November 1988, at the Put-in-Bay site. Sampling procedures, enumeration of plankton samples, and calculation of body mass–length regressions have been provided elsewhere (Berg and Garton 1988), and follow standard methods. Body mass (dry weight) was standardized to the average cladoceran body length and seasonal differences in mass–length regressions were tested using analysis of covariance (ANCOVA) in the SAS General Linear Model (GLM) program (SAS, Inc. 1985).

Results

Within-Species Thermal Tolerance

The two cladoceran species displayed different patterns of thermal tolerance. Mortality of *Bythotrephes* increased with temperature (Fig. 1a). Upper LT_{50}s were nearly identical in summer and autumn (Table 1). Lower LT_{50}s could not be calculated because mortality was lowest at the lowest experimental temperature (Fig. 1a). Individual paired tests for equality of percentages between season at each experimental temperature were significant at 25 and 30°C (Fig. 1a). Autumn mortality was lower than summer mortality at 25°C, but the reverse occurred at 30°C. Despite differences in mortality at 25 and 30°C, *Bythotrephes* did not display significant seasonal thermal acclimation (season × temperature × mortality interaction G-test = 4.80, 4 df, $p > 0.10$). Overall, the positive relationship between 12-h total mortality and temperature was similar in both summer and autumn experiments (Fig. 1a).

In contast to mortality of *Bythotrephes*, 12-h total mortality of *Leptodora* increased with temperature above and below

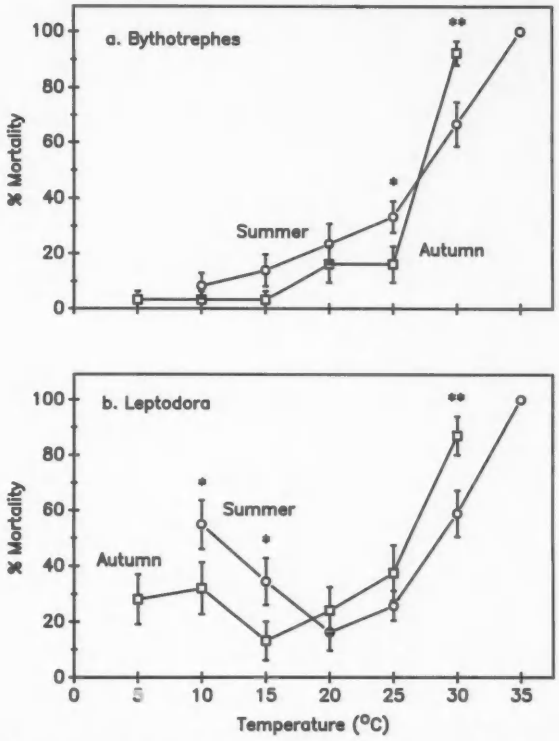

FIG. 1. Percent mortality after 12 h at each experimental temperature, between-season comparison for each species. Vertical bars represent 1 *SD* of the sample mean assuming binomial distribution. Results for test of equality of percentages comparing summer and autumn mortality at each temperature: *, $p<0.05$; **, $p<0.01$.

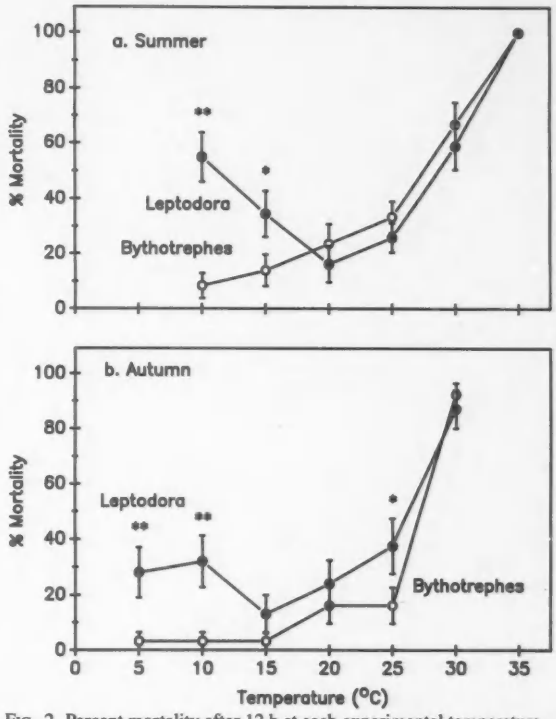

FIG. 2. Percent mortality after 12 h at each experimental temperature, between-species comparison for each season. Symbols same as for Fig. 1.

TABLE 1. Twelve-hour LT_{50}s for *Bythotrephes cederstroemi* and *Leptodora kindti* (°C). Ambient lake temperature at time of summer experiment, 25.0°C; autumn experiment, 10.5°C for *Leptodora* and 7.1°C for *Bythotrephes*. — indicates LT_{50} could not be calculated from the data.

	Upper LT_{50}	*Lower* LT_{50}
Leptodora		
Summer	28.0	9.7
Autumn	24.7	−4.6
Bythotrephes		
Summer	26.2	—
Autumn	25.9	—

ambient temperature (Fig. 1b). For both experiments, mortality–temperature curves were U-shaped and minimum mortality occurred at experimental temperatures near ambient lake temperature. Seasonal acclimation to changing lake temperature was also evident, as the autumn mortality–temperature curve was shifted to the left of the summer mortality–temperature curve (season × temperature × mortality interaction G-test = 13.48, 4 *df*, $p<0.05$) (Fig. 1b). The significant interaction of season and temperature was caused by higher mortality at 10 and 15°C in summer and higher mortality at 25 and 30°C in autumn (Fig. 1b). Upper and lower LT_{50}s were also lower for the autumn experiment than the summer experiment (Table 1).

Between-Species Thermal Tolerance

In summer, *Leptodora* mortality was significantly greater than *Bythotrephes* mortality at experimental temperatures of 10 and 15°C, as revealed by tests for equality of percentages (Fig. 2a). Similarly, mortality of *Leptodora* in autumn was significantly greater than *Bythotrephes* at lower experimental temperatures, 5 and 10°C (Fig. 2b). Mortality at higher temperatures was not significantly different between the two species, except at 25°C in autumn, when *Leptodora* had a significantly higher mortality rate than *Bythotrephes*.

Body Mass–Body Length Regressions

Instars of *Bythotrephes* (Stage I, II, and III) can be readily identified by the number of pairs of lateral spines on the main caudal spine (Ishreyt 1930; Berg and Garton 1988). Growth is discontinuous, complicating calculation of adjusted dry weights from ANCOVA. Therefore body mass–length relationships were analyzed using both ANCOVA and by calculating average dry weight within each instar. *Bythotrephes*, standardized to the same spine length regardless of instar, had greater body mass in summer than autumn (ANCOVA $p<0.0001$) (Fig. 3a). Similarly, mean dry weight for each instar was significantly greater in summer than autumn (ANOVA $p<0.0001$) (Table 2). Mean body mass of Stage I instars was approximately three times greater in summer than autumn, and the magnitude of seasonal

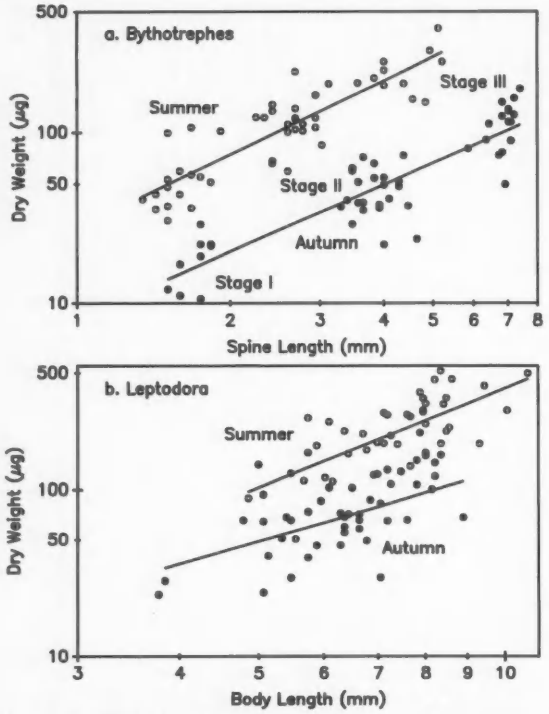

FIG. 3. Dry weight–length regressions for summer and autumn populations. All regressions significant at $p<0.01$. (a) *Bythotrephes*; summer (July 23) regression equation: log(dry weight) = 1.617 + 1.514 (log spine length), $r^2 = 0.76$. Autumn (November 3) regression equation: log(dry weight) = 1.224 + 1.216 (log spine length), $r^2 = 0.60$. (b) *Leptodora kindti*; summer (July 23) regression equation: log(dry weight) = 0.626 + 1.982(log body length), $r^2 = 0.48$. Autumn (October 23) regression equation: log(dry weight) = 0.472 + 1.749(log body length), $r^2 = 0.36$. Adjusted means are significantly different between seasons for each species (ANCOVA, $p<0.001$).

TABLE 2. Mean dry weight (µg) for each instar of *Bythotrephes* from summer (July 23) and autumn (November 3) populations. Values in parentheses represent standard errors about the mean.

	Summer			Autumn		
Instar	Mean dry weight		*N*	Mean dry weight		*N*
I	80.0	(8.2)	16	27.6	(2.6)	10
II	166.3	(10.9)	22	66.3	(4.0)	23
III	302.8	(26.6)	11	154.9	(11.3)	16

differences in mean body mass declined for Stage II and III instars (Table 2).

Size differences between molts were much less for *Leptodora* than *Bythotrephes*, resulting in continuous body mass distribution with increasing length (Fig. 3b). Therefore ANCOVA was appropriate for calculating adjusted dry weights. Body mass of *Leptodora* was significantly greater in summer than autumn (ANCOVA $p<0.0001$) (Fig. 3b).

Fecundity

Fecundity of *Bythotrephes* was greater in autumn than summer (Table 3). Percentage of females carrying asexual broods, mean and per capita brood size were higher in autumn than in summer. A greater number of males were present in summer than autumn, consequently, sexual broods were present in summer but absent in autumn (Table 3). Total reproductive output (sum of per capita sexual and asexual brood size) was three times greater in autumn than summer (Table 3).

Leptodora discharges the contents of its brood pouch when preserved in formalin, therefore fecundity data for this species were unavailable. Sex ratio of *Leptodora* was reversed between summer and autumn, with females more abundant than males in summer, and males outnumbering females in autumn (Table 4).

Abundance–Temperature Relationships

In 1987, *Bythotrephes* abundance was negatively correlated with lake temperature ($r=-0.76$, $p<0.0001$, $n=20$), and peaked in autumn after *Leptodora* abundance had declined (Fig. 4). In 1988, however, *Bythotrephes* abundance showed no significant relationship with lake temperature ($r=0.26$, $p>0.26$, $n=24$), and peak abundance occurred in summer. Overall, combined 1987 and 1988 abundance data for *Bythotrephes* revealed no significant relationship with lake temperature ($r=-0.24$, $p>0.11$, $n=44$) (Fig. 5a).

For 1987 and 1988 combined data, *Leptodora* abundance was positively correlated with lake temperature ($r=0.77$, $p<0.0001$, $n=44$) (Fig. 5b). Maximum abundance occurred in summer, corresponding with peak water temperatures (Fig. 4). The abundances of *Leptodora* and *Bythotrephes* were negatively correlated for 1987 and 1988 ($r=-0.48$, $p<0.001$, $n=44$).

Discussion

The laboratory experiments in this study revealed significant differences in thermal tolerance between the invader, *Bythotrephes*, and ecologically similar native species, *Leptodora*. Specifically, summer and autumn *Leptodora* were less tolerant of cooler temperatures than *Bythotrephes*, and both species were equally tolerant of warmer temperatures. Field data from 1987 suggested that each species might have different thermal optima, with *Leptodora* intolerant of low temperature and *Bythotrephes* intolerant of warm temperature (Berg and Garton 1988). Laboratory results were consistent with 1987 and 1988 seasonal patterns of abundance of *Leptodora* in western Lake Erie. However, *Bythotrephes* was more tolerant of warm temperatures in laboratory experiments than field data from 1987 suggested. Furthermore, in 1988 abundance of *Bythotrephes* peaked in midsummer, at temperatures of 23–25°C, rather than in autumn as in 1987. Therefore, factors other than intolerance to warm temperatures limit abundance of *Bythotrephes* in western Lake Erie in summer.

A positive relationship between mortality and temperature is characteristic of coldwater stenotherms. *Bythotrephes cederstroemi* occurs in cool, deepwater oligotrophic lakes in northern Europe (Stenson 1972; Lehman 1987). However, the influence of temperature on distribution and seasonal population abundance of *B. cederstroemi* has not been well studied; most of the European research on *Bythotrephes* has dealt with a related species, *B. longimanus*. Even these few reports, however, offer conflicting conclusions regarding thermal preferences of *Bythotrephes*. *B. longimanus* has a wide geographic range, encompassing many different thermal environments, throughout

TABLE 3. Demographic profile of summer and autumn populations of *Bythotrephes cederstroemi* from western Lake Erie in 1988. Summer sample collected July 23; autumn samples November 3. Abundance represents the range for entire season; values in parentheses are standard error about the mean.

	Summer	Autumn
Abundance range (No./m^3)	0–102.1	0–10.1
Females		
Stage I	53	47
II	47	45
III	33	43
Total	133	135
Males		
Stage I	16	8
II	21	8
III	0	0
Total	37	16
Percent females with asexual broods	60.2	83.7
Mean asexual brood size	2.98 (1.10)	7.27 (1.26)
Per capita asexual brood size	1.40 (1.69)	5.38 (3.42)
Percent females with sexual eggs	1.5	0
Mean sexual brood size	4.0 (0.0)	0
Per capita sexual brood size	0.07 (0.10)	0
Combined asexual and sexual per capita reproductive effort	1.47 (1.79)	5.38 (3.42)
Adjusted mean dry weight (μg)	192.5 (11.1)	57.7 (3.4)

TABLE 4. Demographic profile of summer and autumn populations of *Leptodora kindti* from western Lake Erie in 1988. Summer sample collected July 23; autumn sample October 23. Abundance represents the range for entire season; values in parentheses are standard error about the mean.

	Summer	Autumn
Abundance range (No./m^3)	51.9–398.5	0.3–120.7
Females	145	36
Males	6	115
Adjusted mean dry weight (μg)	191.5 (13.1)	85.5 (5.5)

southcentral Europe, and is sympatric with *B. cederstroemi* in western Russia and Finland (Ishreyt 1930). *B. longimanus* has been characterized as a "summer" form, present in the plankton from late May through late October, with males and sexual resting eggs being produced in autumn (Andrew and Herzig 1984; Herzig 1985). However, in the Rybinsk Reservoir, Russia, Mordukhai-Boltovskaia (1956) reported that both *B. cederstroemi* and *B. longimanus* were found in successively deeper, cooler water as temperature increased in surface waters during the summer, evidence that these species could not tolerate peak summer water temperatures. Ishreyt (1930) postulated that different "kolonies" (= physiological races?) of *B. longimanus* were distributed across Europe, and distinguished two major forms; the first, *B. longimanus* sensu strictu, as eurythermal and limnetic, and the second, *B. longimanus* var. *arcticus*, as coldwater stenothermal and littoral. Lastly, there has been some doubt cast on the validity of the distinction between *B. cederstroemi* and *B. longimanus* (reviewed in Evans 1988), which may explain some of the conflicting reports on thermal preferences in the European literature. Patterns of seasonal abundance and sexual reproduction, thermal tolerance in the laboratory, and lack of population genetic variability support the hypothesis that North American *B. cederstroemi* originated

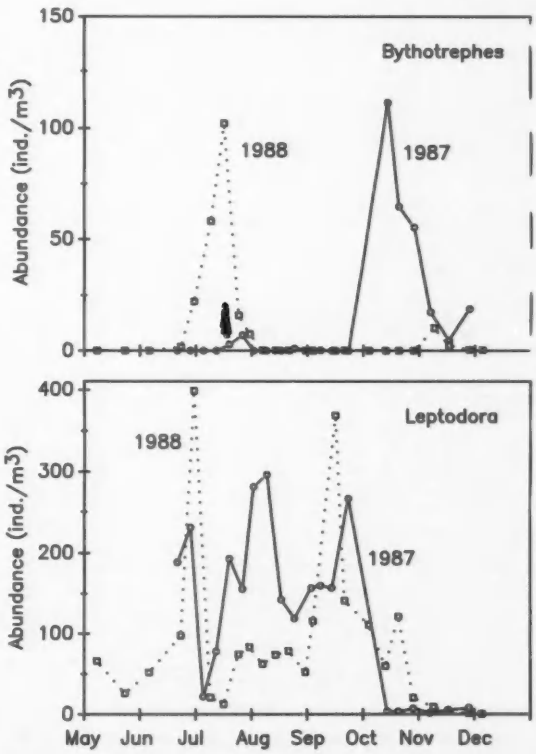

FIG. 4. Mean abundance of *Bythotrephes* and *Leptodora* near South Bass Island, western basin of Lake Erie during 1987 and 1988. Data for 1987 from Berg and Garton (1988).

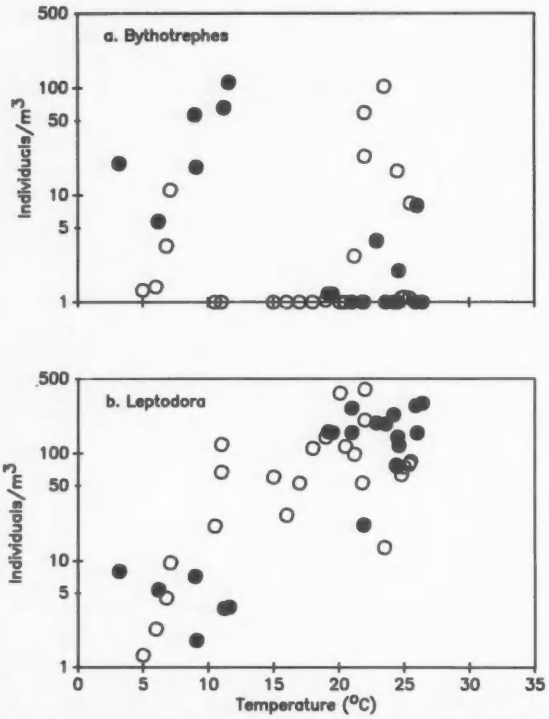

FIG. 5. Predatory cladoceran abundance ($\log(x+1)$ transformed) plotted against water temperature for 1987 (closed circles) and 1988 (open circles). (a) *Bythotrephes cederstroemi*, $r=-0.24$, $n=44$, $p>0.11$. (b) *Leptodora kindti*, $r=0.77$, $n=44$, $p<0.0001$.

from a single, northern European, stenothermal coldwater population.

Seasonal adjustment of physiological processes in response to changing temperature can result from either phenotypic plasticity (i.e. acclimation) of fixed genotypes, or, in asexual species with short generation times, changes in genotypes present in the population via clonal replacement. *Bythotrephes* has only recently invaded the Great Lakes, and as a consequence has little genetic variability and lacks genetic differentiation among populations within each lake (Garton and Berg 1990; D. J. Berg and D. W. Garton, unpubl. data). In the case of Lake Erie *Bythotrephes*, all possible clones would have a very high degree of genetic similarity. In *Daphnia*, acute thermal tolerance varies among genetically distinct clones derived from habitats with different peak summer temperatures (MacIsaac et al. 1985). Seasonal clonal succession in populations of *Daphnia magna* also results from genotype-dependent responses to changing water temperature (Carvalho 1987). The absence of seasonal acclimation in *Bythotrephes* may result from lack of interclonal genetic variability, or simply lack of clonal replacement because summer and autumn populations represent identical clones.

In contrast, native *Leptodora* has appreciable levels of within-population genetic variability in Lake Erie, and allele frequencies vary throughout the year (D. J. Berg and D. W. Garton, unpubl. data). Thermal tolerance and mortality rate of *Leptodora* showed seasonal acclimation responses, with upper and lower LT_{50}s shifted to lower temperatures in autumn than in summer. The pattern of mortality rates also showed seasonal effects. These strong seasonal acclimation responses are evidence that either phenotypic plasticity is related to individual genetic variability per se, or that clonal replacement occurs in *Leptodora*.

Mean dry weight, adjusted for differences in body length, provides an index of physiological condition independent of population abundance. Both *Bythotrephes* and *Leptodora* adjusted mean weights were positively correlated with population abundance in 1988. Mean adjusted dry weights were significantly greater in summer (high abundance) than autumn (low abundance). Likewise, in 1987, *Bythotrephes* weighed more during periods of peak abundance (autumn) than low abundance (summer) (Berg and Garton 1988). Adjusted mean dry weight may reflect the availability of prey or absence of intra- and interspecific competition for available prey. In western Lake Erie, peak abundance of potential prey (e.g. *Daphnia, Bosmina,* and *Eubosmina*) occurs in early summer and early autumn (Phipps 1987). Prey abundance declines in midsummer and late autumn (Phipps 1987), periods when abundance of predatory cladocerans also declines (midsummer), and when predator adjusted dry weight declines (autumn) (Berg and Garton 1988).

Although adjusted mean dry weight was correlated with abundance in *Bythotrephes* in 1987 and 1988, fecundity was not. Fecundity (average and per capita brood size) was significantly lower in summer than autumn for both years (Berg and Garton 1988, this study). Although *Bythotrephes* can tolerate summer temperatures in Lake Erie, it may be unable to fully acclimate (i.e. reduce metabolic rate) at higher environmental temperature. Increased maintenance metabolic rate, associated with increased temperature, results in less energy available for reproduction, and hence reduced fecundity. In the case of *Bythotrephes*, abundance and adjusted mean weight under specific environmental conditions did not reflect differences in individual fecundity in summer and autumn populations.

The appearance of males and sexual resting eggs occurs when parthenogenetic cladoceran populations exist near environmental limits. For *Bythotrephes*, males were more frequent in summer than autumn, and sexual eggs were present only in summer samples (Table 3). The pattern was reversed for *Leptodora*, with more males present in autumn than summer (Table 4), although data on sexual eggs were lacking. Andrews (1948) and Cummins et al. (1969) both reported similar relative abundance patterns for *Leptodora*, with males greatly exceeding 50% of the population in late September and October.

Leptodora and *Bythotrephes* can be characterized as eurythermal, with both species present in western Lake Erie over a wide range of water temperatures (approximately 4–27°C). However, abundance of *Leptodora* was directly related to temperature (Berg and Garton 1988, this study). *Leptodora* has been previously reported as temperature-limited, disappearing at water temperatures below 14°C (Andrews 1984; Cummins et al. 1969). Poor tolerance of *Leptodora* to low temperature in laboratory experiments can explain reduced abundance and perhaps why it was succeeded by *Bythotrephes* in autumn of 1987 in western Lake Erie. However, thermal sensitivity of *Bythotrephes* cannot explain numerical dominance of *Leptodora* in western Lake Erie in summer. In the laboratory, both species tolerate warm summer temperatures (20–30°C) equally well, and in 1988 peak abundances of both species occurred in midsummer (Fig. 4).

Other factors potentially responsible for low abundance of *Bythotrephes* during summer months include (1) competitive exclusion by *Leptodora*, (2) prey availability, and (3) fish pre-

dation. *Bythotrephes* may be nearing an environmental limit during warm summer months in western Lake Erie, placing it at a competitive disadvantage to *Leptodora*. Evidence that *Bythotrephes* nears upper thermal limits in summer include reduced fecundity and the presence of males and resting eggs. In addition, in Lake Erie for both 1987 and 1988 *Bythotrephes* was usually much less abundant than *Leptodora* during summer months; likewise *Leptodora* has been reported as a numerical dominant to *Bythotrephes* in some European lakes (Mordukhai-Boltovskaia 1956; de Bernardi and Guissani 1975; Herzig 1985). Therefore, *Bythotrephes* may be unable to exploit abundant resources in the summer while *Leptodora* is present.

Abundance of common potential prey declines in mid-summer (Phipps 1987) resulting in population declines of both predators (Berg and Garton 1988; this study). A shift in prey size is associated with declining prey numbers (Culver 1980; Culver et al. 1985; T. Phipps and D. A. Culver, Ohio State University unpubl. data). The prey capturing appendages of *Bythotrephes* are more robust than those of *Leptodora*, therefore shifts in size and abundance of prey may favor one predator over another.

Seasonal abundance of *Bythotrephes* may also be dependent on fish predation. Population abundance of both species decline in midsummer, but subsequently only *Leptodora* recovers to predecline abundance. Selective predation by various species of fish has been reported on *B. longimanus* and *B. cederstroemi* in European lakes (Stenson 1972; di Bernardi and Guissani 1975). In Lake Erie, *B. cederstroemi* is prey for yellow perch (*Perca flavescans*), white perch (*Morone americana*), walleye (*Stizostedion vitreum*), and white bass (*M. chrysops*) (Bur et al. 1986), and in Lake Superior is consumed by pink (*Oncorhyncus gorbuscha*) and chinook salmon (*O. tshawytscha*) (Cullis and Johnson 1988). In small lakes, fish predation prevents *Bythotrephes* from reaching high abundance, and large populations only occur when predation pressure is relaxed (Stenson 1972; Nilsson 1979). The opaque body of *Bythotrephes* may lead to greater vulnerability to fish predation than transparent *Leptodora*.

Although declining water temperature can explain *Leptodora* being succeeded by *Bythotrephes* in 1987, elucidating the mechanism(s) preventing *Bythotrephes* from being more numerous during the summer in western Lake Erie will require additional study on prey preference, feeding rate, seasonal patterns of prey availability and fish predation on these two species, as well as additional study of relationships between genetic variability and phenotypic plasticity of invading species in novel habitats.

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Serum Lipoproteins in Striped Bass (*Morone saxatilis*): Effects of Starvation

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Withholding food for 4 wk resulted in more extensive alterations within serum lipoprotein classes in striped bass (*Morone saxatilis*) than were evident in serum total lipids where only a decrease in triglycerides was significant. Total serum lipoprotein concentrations were about 3300 mg/dL in both fed and starved fish; however, high density lipoproteins (HDL) increased and very low density lipoproteins (VLDL) decreased significantly in the starved group. Quantitatively, the greatest changes in VLDL, the primary transporter of triglycerides, were declines in triglycerides and cholesterol esters. Within low density lipoproteins, triglycerides and phospholipids decreased. The HDL composition was affected least by starvation. Phospholipids were the dominant lipid in HDL and increased from 479.1 mg/dL in fed to 670.2 mg/dL in starved fish. The apoproteins of each lipoprotein class changed little qualitatively due to starvation. Our results reflect the importance of maintaining high levels of lipoproteins as a transport system for lipids from storage depots to depleted tissues permitting survival during prolonged periods of starvation that are often encountered by fish during colder months and during spawning migrations. Furthermore, during starvation HDL appears to assume increased significance as a vehicle for the transport of structural lipids to maintain tissue integrity.

La privation de nourriture pendant quatre semaines a produit des altérations plus importantes dans les classes de lipoprotéines sériques chez le Bar d'Amérique (*Morone saxatilis*) qu'il ne paraissait dans les lipides sériques totaux, ce qui n'avait révélé de baisse importante que dans la teneur en triglycérides. La concentration en lipoprotéines sériques totales était d'environ 3300 $mg \cdot dL^{-1}$ chez les sujets nourris ainsi que les sujets affamés. Cependant, la teneur en lipoprotéines de haute densité (HDL) a augmenté alors que celle des lipoprotéines de très basse densité (VLDL) a diminué significativement chez les sujets affamés. Sur le plan quantitatif, les plus importants changements en VLDL, le principal vecteur des triglycérides, a été la diminution en concentration des triglycérides et des esters du cholestérol. Quant aux lipides de basse densité (LDL), la teneur en triglycérides et en phospholipides a diminué. La composition en HDL a le moins varié par suite du traitement. Les phospholipides constituaient la principale forme de HDL et leur concentration est passée de 479,1 $mg \cdot dL^{-1}$ chez les sujets nourris à 670,2 $mg \cdot dL^{-1}$ chez les sujets affamés. Les apoprotéines de chaque classe de lipoprotéines ont peu varié qualitativement par suite de l'inanition. Nos résultats montrent l'importance de garder les lipoprotéines à un niveau élevé comme moyen de transport des lipides, des réserves aux tissus qui se sont appauvris, ce qui assure la survie durant les périodes prolongées de famine que les poissons doivent souvent affronter durant les mois d'hiver et au cours des migrations pour la reproduction. En outre, le HDL associé à l'inanition semble gagner en importance comme vecteur pour le transport des lipides structuraux en vue de conserver l'intégrité des tissus.

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Fish have an impressive ability to withstand long periods of starvation. This capacity is adaptive since many species of fish are subjected to natural periods of starvation or very low food intake during spawning or cold months of the year in temperate latitudes when prey abundance is low (Love 1970). Energetic demands are met primarily by lipid oxidation in fish (Cowey and Sargent 1979) and this is particularly true during starvation (Shul'man 1972).

Lipids are stored in substantial quantities in various tissues, including muscle, liver, and mesenteric depots, and, in addition

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to being a source of energy, provide components for membrane synthesis and precursors of steroid hormones. Many studies have shown that fish mobilize energetic lipids (e.g. triglycerides) from tissues preferentially to structural lipids (e.g. phospholipids) during starvation, but phospholipids are mobilized if starvation is prolonged (reviewed by Love 1980; Henderson and Tocher 1987). Since a basic minimum of energy and structural integrity of tissues must be maintained for survival, lipids must be supplied to depleted tissues from storage depots. Beyond these needs for maintenance, substantial quantities of energetic and structural lipids must be supplied to gonads, especially ovaries, during reproductive development. In anadromous species which undergo lengthy spawning migrations without feeding, gonadal development is dependent to a large extent on the transport of stored lipids (Henderson and Tocher 1987).

Lipids are transported in blood serum complexed with specific proteins (apolipoproteins) as particles known as lipoproteins. Serum lipoproteins are routinely characterized by size, density, and their chemical composition. In ascending order of density, they are chylomicrons, very low density (VLDL), low density (LDL), and high density (HDL) lipoproteins. Although studied rather extensively in birds and mammals, serum lipoproteins have been described in only a few species of fish (Chapman 1980), most notably rainbow trout (Chapman et al. 1978; Skinner and Rogie 1978; Fremont et al. 1981). A common feature of serum lipoproteins in teleost species studied to date is that they are found in very high concentrations relative to birds and mammals (Chapman 1980). Moreover, during the prespawning period HDL appears to become elevated in rainbow trout (Fremont and Marion 1982) and pink salmon (Nelson and Shore 1974).

From the above observations, assessing changes in serum lipoproteins during starvation may reveal which components are most significant for maintenance of tissue integrity and synthesis. Black and Skinner (1986) found that starvation produced changes in cholesterol concentrations in serum lipoproteins of rainbow trout. However, there are no published reports on the effects of fasting on the lipid and protein compositions of serum lipoproteins in fishes. To investigate this, a study was conducted to determine the changes in the composition and concentration of serum lipoprotein classes of starved striped bass (*Morone saxatilis*), an anadromous species that experiences periods of starvation during winter months and during spawning (Woodhull 1947; Stevens 1966; Jones and Sidell 1982).

Materials and Methods

Adult striped bass (4-yr-old, mean FL 47 ± 15 cm) were collected in June 1983, after the spawning period, from the San Francisco Bay estuary by gill net and transported to a 20 000-L raceway using an aerated mobile fish transporter. They were acclimatized in the raceway to ambient, filtered bay water (approximately 17°C and 25 ‰ salinity) for 2 wk prior to randomized placement in the experimental tanks. All fish were fed anchovies on alternate days while in the raceway. Each circular 3200-L fiberglass experimental tank contained six fish and received flow-through ambient filtered bay water (approximately 18.5°C and 27 ‰ salinity) under the natural photoperiod. During the 4-wk experimental period, fish in one of the tanks were fed anchovies daily until satiated whereas no food was provided to fish in the other tank.

Blood was obtained by cardiac puncture using sterile Vacutainers[3] without anticoagulant and allowed to clot prior to centrifugation and serum withdrawal. After separation, each serum sample received antibacterial agents (0.001% sodium merthiolate and 0.01% sodium azide) and 1 mg/mL EDTA. Samples were stored at −80°C under nitrogen until analyzed.

Isolation of Lipoproteins

Serum lipoproteins were separated by sequential ultracentrifugation into density classes routinely used for human lipoprotein isolation for comparative purposes with studies of fishes and other animals. The density intervals employed were; $d<1.0063$ g/mL for VLDL, $1.0063<d<1.063$ g/mL for LDL, and $1.063<d<1.21$ g/mL for HDL. Appropriate density salt solutions for ultracentrifugal isolation of lipoprotein classes were prepared with NaCl and NaBr in deionized water and checked by pycnometry according to Lindgren (1975). All salt solutions contained antibacterial and antioxidation agents at the same concentrations as in serum samples. Since the nonprotein density of striped bass serum was unknown, it was adjusted to 1.0063 g/mL by exhaustive dialysis with a NaCl solution of $d = 1.0063$ g/mL prior to lipoprotein fractionation.

Lipoprotein fractions were isolated by the sequential ultracentrifugation techniques described in detail by Lindgren (1975) using a Beckman L5-75 with a 50-Ti rotor at 10°C. Briefly, 2.0 mL of dialyzed serum was added to a polycarbonate centrifuge tube and mixed with 4.0 mL of d 1.0063 g/mL NaCl solution and spun at 45 000 rpm (135 000 × g) for 18 h. The top 1.0 mL containing VLDL and the next 1.0 mL background fraction were removed. The remaining solution's density was raised to 1.063 g/mL with 2.0 mL of the appropriate density NaCl–NaBr solution and centrifuged for 20 h at 45 000 rpm (135 000 × g). The top 1.0 mL containing LDL and the next 1.0 mL for background assessment were removed. The remaining infranatant was increased in density to 1.21 g/mL with 2.0 mL of a NaCl–NaBr solution and spun at 45 000 rpm (135 000 × g) for 40 h at 10°C. As before, the top 1.0 mL containing HDL was removed as well as the next 0.5 mL for background.

Lipid Composition Analysis

Lipids were extracted from aliquots of serum and lipoprotein fractions with 2 × 15 volume of ethanol–ether (3:1). Precipitated protein was removed by centrifugation and the supernatant was evaporated under a stream of nitrogen. The dried lipid residue was reconstituted with chloroform–methanol (1:1) and analyzed for total lipid (Harvey and Patton 1981) and lipid class composition in triplicate using an Iatroscan TH-10 analyzer (Iatron Laboratories, Inc., Tokyo, Japan). The S-II Chromarods were developed in a solvent system of hexane–ethyl ether–acetic acid (95:5:0.2) which resolves triglycerides, alkyl esters/glyceryl ethers, free cholesterol, cholesterol esters, and phospholipids. Triglycerides were also determined in lipoprotein fractions and background fractions by the enzymatic method of Bucolo and David (1973). Lipid-containing solutions were always stored in Teflon-lined screw-capped culture tubes under nitrogen in the dark at −20°C between procedural manipulations.

[3]References to trade names does not imply endorsement by the National Marine Fisheries Service.

TABLE 1. Total concentrations of lipid components and protein in serum of striped bass fed or starved for 4 wk. Values represent mean ± SE of six fish expressed as mg/dL. * = significantly different from fed ($P<0.05$).

Component	Treatment	
	Fed	Starved
Triglycerides	618 ± 132	252 ± 53*
Glyceryl ethers/alkyl esters	150 ± 60	189 ± 88
Cholesterol esters	240 ± 46	231 ± 48
Free cholesterol	201 ± 28	202 ± 29
Phospholipids	961 ± 118	859 ± 163
Total lipids	2169 ± 215	1733 ± 298
Protein	4067 ± 228	4433 ± 208

Apoprotein Analysis

Total protein concentrations in serum, lipoprotein fractions, and background fractions were determined by a modified Lowry method for lipoprotein samples, which incorporated sodium dodecyl sulphate into the alkali reagent, allowing direct assay without delipidation (Markwell et al. 1981). Bovine serum albumin standards were employed.

Apoproteins within each lipoprotein fraction were separated and characterized by molecular weight according to the sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) method of Laemmli (1970). Samples of apo-VLDL, apo-LDL, and apo-HDL were applied to separate gels to resolve high and low molecular weight apoproteins. High molecular weight apoproteins were separated using a 3.0% monomer stacking gel atop a 5.0% separation gel and low molecular weight proteins were resolved with a 5.0% monomer stacking gel and a 10.5% separation gel. Proteins were fixed and stained in a 0.1% Coomassie Brilliant Blue solution in 50% acetic acid, prepared fresh daily. Gels were destained by repeated washings in 7% acetic acid, dried, and scanned in a Helena "Fluor-vis" densitometer (Helena Laboratories, Inc., Beaumont, TX) equipped with a 560-nm filter. The molecular weight of each electrophoretic band was determined by comparing its mobility with that of a series of SDS-PAGE protein standards purchased from Bio-Rad Laboratories, Richmond, CA, according to the formula of Weber and Osborn (1969). Apoprotein electrophoretic bands were not quantified for reasons discussed extensively by Chapman et al. (1975).

Statistical Analysis

Differences in the lipid compositon of lipoprotein fractions and serum from fed and starved striped bass were analyzed using the paired *t*-test.

Results

Each treatment group of striped bass contained four females and two males. Statistical analysis of the data revealed no differences in lipid composition attributable to sex. Therefore, data from both sexes were combined.

Withholding food for 4 wk resulted in a 20% decrease in serum total lipids, but only the decline in triglycerides was statistically significant (Table 1). Serum triglyceride levels decreased by about 60%. Essentially no changes occurred in the concentrations of free cholesterol, cholesterol esters, and unknown compounds referred to here as glyceryl ethers/alkyl esters due to their similar mobilities on the Chromarods. The glyceryl ethers/alkyl esters fraction may contain 1-0-alkyl-diacylglycerides which have been found in other fish species (Mori et al. 1972; Kayama 1975; Mills and Taylaur 1977). Serum phospholipids were decreased by 11% and total serum protein increased by 9% in fish subjected to starvation, but neither change was statistically significant.

Mean weight percentage composition of the major lipoprotein classes revealed that starvation altered the distributions of lipids and protein in all lipoprotein fractions (Table 2). The predominant lipid in striped bass VLDL was triglyceride, accounting for about half of the lipid present in fed fish and 39% in starved fish. Protein contributed, by weight, 9.8% of the VLDL in fed striped bass. The proportion of protein in VLDL increased during the starvation period of 18.1%. This was due to a much greater depletion of lipid relative to protein in VLDL of starved fish compared with fed; the mean absolute concentrations of protein in fed and starved striped bass VLDL were approximately the same, 62.7 mg/dL and 75.0 mg/dL, respectively. Within the lipids, the quantitative decrease in VLDL triglycerides, from 445.2 mg/dL in fed fish to 159.9 mg/dL, was the major change observed due to starvation and was largely responsible for the statistically significant decrease in total lipid (Table 2). Starvation produced a greater mean percent contribution of phospholipids in VLDL due to the decreased contribution of triglycerides, despite little change in concentration (125.7 mg/dL in fed fish, 114.1 mg/dL in starved fish). Cholesterol esters were diminished six-fold and lipids within the glyceryl ethers/alkyl esters fraction four-fold in percent composition in VLDL of starved fish. The percent contribution of cholesterol to VLDL particles remained essentially unchanged.

There was a greater proportion of cholesterol, in free and esterified forms, in striped bass LDL than in other lipoprotein classes; however, triglycerides predominated in the LDL of fed fish (Table 2). Protein contributed, on a mean weight basis, a greater proportion of the LDL mass than in VLDL. Consequently, there was less lipid in LDL particles than in VLDL. In fed fish, phospholipids comprised about the same proportion of LDL as for VLDL (14.1 and 18.6%, respectively) and also on a mean concentration basis (138.4 mg/dL in LDL and 125.7 mg/dL in VLDL). There was an inverse relationship between cholesterol esters and free cholesterol in LDL compared to VLDL, with cholesterol esters predominating in LDL.

Starvation resulted in significant decreases in the mean weight percentages of triglycerides, phospholipids, and total lipid in striped bass LDL (Table 2). The other lipid components and protein increased in starved fish LDL, although the changes were not statistically significant. Compounds with chromatographic mobilities similar to glyceryl ethers and alkyl esters accounted for a greater proportion of LDL in fed and starved striped bass than in the other two lipoprotein fractions.

Phospholipids dominated striped bass HDL lipids and accounted for about 33.5% of the weight of HDL in fed and starved fish (Table 2). In concentration, however, phospholipid increased from 479.1 mg/dL in fed fish to 670.2 mg/dL in starved fish. The composition of the HDL fraction appeared to be affected the least by starvation. The only significant change occurred in the triglycerides fraction which comprised a small proportion of HDL.

Summation of all lipid components and protein concentrations within each lipoprotein class provided an estimate of the concentration of individual lipoprotein classes within each fish.

TABLE 2. Mean weight percent composition of lipid components and protein in serum lipoprotein classes of striped bass fed or starved for 4 wk. Values represent mean (SE) of six fish. * = significantly different from fed ($P<0.05$); ** = significantly different from fed ($P<0.01$); *** = significantly different from fed ($P<0.001$).

	VLDL		LDL		HDL	
Component	Red	Starved	Fed	Starved	Red	Starved
Triglycerides	50.6	38.8	27.2	6.5**	4.6	1.2***
	(9.3)	(5.6)	(4.6)	(1.1)	(0.6)	(0.1)
Glyceryl ethers	4.9	1.2	9.1	15.1	0.1	0.8
/alkyl esters	(4.2)	(1.2)	(3.4)	(3.5)	(0.1)	(0.6)
Cholesterol	6.9	1.1*	12.2	15.6	4.3	3.2
esters	(2.5)	(0.8)	(2.9)	(0.9)	(1.0)	(1.0)
Free cholesterol	10.2	11.5	8.4	11.5	2.9	2.5
	(3.5)	(8.5)	(1.2)	(5.0)	(0.3)	(0.8)
Phospholipids	18.6	29.3*	14.1	9.1**	33.4	33.9
	(4.0)	(2.1)	(1.1)	(0.8)	(1.2)	(2.8)
Total lipids	91.2	81.9*	71.0	57.8*	45.3	41.6
	(2.0)	(3.4)	(4.0)	(3.5)	(1.3)	(4.0)
Protein	9.8	18.1*	29.0	42.2	54.7	58.4
	(2.5)	(3.3)	(4.9)	(3.7)	(1.3)	(4.0)

TABLE 3. Concentrations and mean weight percent of serum lipoprotein classes in striped bass fed or starved for 4 wk. Values represent mean (SE) of six fish. *significantly different from fed ($P<0.05$; **significantly different from fed ($P<0.01$); ***significantly different from fed ($P<0.001$).

	VLDL		LDL		HDL		Σ LP
Treatment	mg/dL	wt %	mg/dL	wt %	mg/dL	wt %	mg/dL
Fed	834	25.1	938	28.0	1436	46.9	3208
	(147)	(2.0)	(175)	2.1)	(65)	(3.8)	(378)
Starved	401*	11.7***	1063	30.9	1977**	57.4*	3441
	(35)	(0.9)	(98)	(2.8)	(101)	(2.4)	(91)

Although mean total lipoprotein concentrations changed little between fed and starved striped bass, significant differences occurred within lipoprotein classes (Table 3). The HDL was the most abundant lipoprotein in striped bass serum and was significantly elevated in starved fish. Inversely, the least abundant lipoprotein, VLDL, declined significantly in concentration subsequent to starvation. This was due, primarily, to the depletion of triglycerides, and to a lesser extent by lower levels of cholesterol esters and cholesterol. The LDL comprised about 30% of the lipoproteins and the concentration remained essentially unchanged by starvation.

The apoproteins of VLDL and LDL were resolved in sodium dodecyl sulphate polyacrylamide gels of 5% monomer concentration and those of HDL in gels of 10.5% monomer (Fig. 1). Apo-VLDL displayed four bands. The predominant component remained near the origin of the separating gel and was a broad, intense band with a molecular weight greater than 250 000 daltons. The remaining bands were much narrower and fainter and contained proteins of approximately 207 000, 47 000, and 42 000 daltons. No apoproteins with molecular weights less than 42 000 were evident on any gels of apo-VLDL.

The apoproteins of LDL separated into five major bands and two bands containing trace amounts of dye-binding material. As with apo-VLDL the largest, darkest staining component had a molecular weight in excess of 250 000 daltons. Apo-LDL also contained a band at about 42 000 daltons with the same staining characteristics as seen for apo-VLDL. Other bands of similar size and intensity were observed with molecular weights of about 77 000, 60 000, and 51 000 daltons. Trace amounts of polypeptides occurred with apparent molecular weights of approximately 203 000 and 149 000 daltons.

Apo-HDL was distinctly different in composition relative to VLDL and LDL (Fig. 1). The largest and darkest staining component corresponded to a molecular weight of about 24 000 daltons. The next most prominent band had an apoprotein of 13 000 daltons. A substantial amount of material was observed with a molecular weight greater than 100 000 daltons. Other faint bands were identified with molecular weights of 76 000, 50 000, and 28 000 daltons.

Qualitatively, withholding food for 4 wk resulted in very little change in the apoprotein content of lipoproteins in striped bass. Within the resolution obtained by SDS-PAGE, all components observed on the gels for apo-HDL and apo-LDL from fed fish were present on those from starved fish. The only difference noted occurred in apo-VLDL, where the band with an estimated molecular weight of 207 000 daltons found in fed fish was absent in apo-VLDL of starved fish. Since analytical manipulations (e.g. repeated washings, dialysis, and ultracentrifugations of lipoprotein fractions) and variable chemical characteristics (e.g. differential chromogenicities to stains between electrophoresis bands) are known to compromise the validity of band quantification (Herbert et al. 1978), we did not assess apoprotein concentrations within lipoprotein fractions of fed or starved fish.

Discussion

Serum Lipids

A decrease in triglycerides concentration was the only statistically significant change observed in the serum of starved

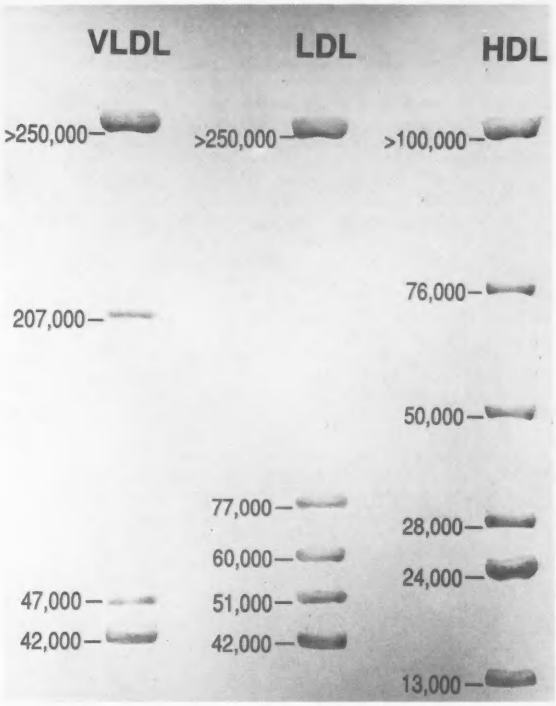

FIG. 1. Representations of sodium dodecyl sulphate-polyacrylamide gels of striped bass apolipoproteins. Apo-VLDL and apo-LDL were electrophoresed in 5% monomer gels and apo-HDL in 10.5% monomer gels. Gels were stained with Coomassie Briliant Blue and molecular weights were calculated from a series of molecular weight protein standards.

striped bass. This decline was not unexpected, however, since it is known that during the initial phases of starvation in fish triglycerides are catabolized while other lipids remain largely unchanged (Love 1980). Also, it has been shown in pink salmon (*Oncorhynchus gorbuscha*) that, during the nonfeeding spawning migration, the liver's triglyceride synthetic ability is lost and blood triglyceride levels fall (Phleger 1971). Decreased serum or plasma triglycerides have been observed in (*Oncorhynchus mykiss* (formerly *Salmo gairdnerii*; Takashima et al. 1971), *Salmo alpinus* (Dannevig and Norum 1983), and *Pleuronectes platessa* (White and Fletcher 1986) after periods of starvation.

Serum Lipoproteins

All the major lipid components were found to a variable extent in each serum lipoprotein class. Variability in the concentrations of lipid components between fish within each treatment group was high and has been noted in other fish species (Chapman et al. 1977; Fremont et al. 1981; Farrell and Munt 1983).

The lipid and protein composition of lipoprotein classes in the serum of fed striped bass was generally similar to that found in rainbow trout (Chapman et al. 1978; Skinner and Rogie 1978; Fremont et al. 1981). In comparison with rainbow trout, striped bass VLDL was somewhat richer in triglycerides and depleted in cholesterol esters. The LDL of striped bass contained the most total cholesterol (free + esterified) of all the lipoprotein classes; however, triglycerides were the predominant lipid in fed striped bass LDL. Phospholipids accounted for the greatest proportion of striped bass HDL lipid, consistent with other fish species studied (Chapman 1980). Otherwise, the HDL of fed striped bass was composed of similar proportions of triglycerides and cholesterol, lower cholesterol esters, and greater protein in relation to the composition of HDL in salmonids (Nelson and Shore 1974; Chapman et al. 1978; Skinner and Rogie 1978; Fremont et al. 1981).

The apoproteins of striped bass lipoprotein classes were characterized only by their molecular weights. This technique, by itself, does not constitute definite identity but is often used for comparative purposes between lipoprotein classes or with data from other species. In the SDS-PAGE of striped bass VLDL and LDL, the apoprotein band with a molecular weight >250 000 daltons was by far the largest and most intensely stained and may be analogous to mammalian apo-B (Eisenberg 1979; Steinberg 1979). Electrophoretic separations of VLDL and LDL from other fish species have found a similar apoprotein which, in combination with immunological and chemical analyses, has been determined to resemble apo-B (Mills et al. 1977; Chapman et al. 1978). Striped bass VLDL and LDL contained several other apoproteins of lower molecular weights in lesser amounts. This was also the case with rainbow trout (Chapman et al. 1978; Skinner and Rogie 1978). The HDL of striped bass differed from VLDL and LDL in that the primary apoproteins had molecular weights of about 24 000 and 13 000 daltons, and based on these weights may be similar to human apo-AI (27 000 daltons) and apo-AII (17 000 daltons) (Schaefer and Levy 1979). Rainbow trout HDL was found to contain predominantly apoproteins characteristic of apo-AI and apo-AII (Chapman et al. 1978; Skinner and Rogie 1978). The results of the present study and those from studies of fish, birds (Chapman et al. 1977), and mammals (Hollanders et al. 1986) would suggest that the apolipoprotein composition of higher vertebrates is generally similar.

The concentration of total serum lipoproteins in both fed and starved striped bass was high, on the order of that found in salmonids (Nelson and Shore 1974; Chapman et al. 1978; Skinner and Rogie 1978), and reflects the importance of lipids in fish metabolism. Although the total serum lipoprotein concentration was unchanged by starvation, the relative contribution of lipoprotein classes was affected such that VLDL declined and HDL increased. Further, the changes in lipid distributions within serum lipoprotein classes of starved striped bass indicate that there were greater adjustments of lipid metabolism and transport produced by starvation than would be evident by simply assessing lipid changes in serum.

The largest change, on a mean weight percent basis, in the VLDL and LDL of starved striped bass was the depletion of triglycerides. Since VLDL is the principal transport vehicle for triglycerides (Eisenberg 1979) and LDL is formed from VLDL (Brown and Goldstein 1986), it may be expected that the combined influences of no ingestion, depletion of body reserves, and reduced triglyceride synthesis would result in decreased VLDL and LDL triglycerides.

In starved striped bass, HDL composition was the least affected of the serum lipoproteins. The HDL have been shown to be involved in "reverse" cholesterol transport, from peripheral tissues back to hepatocytes (Miller et al. 1985), and cholesterol and phospholipid exchanges between lipoproteins and

tissues (Glomset 1979). Cholesterol and cholesterol ester values were unaffected by starvation in striped bass HDL, both in mean weight percent and concentration. These results are similar to those of Black and Skinner (1986), using cholesterol concentration as an estimator of lipoprotein concentrations in rainbow trout. They found that 8 wk starvation produced no change in HDL cholesterol but diminished VLDL and LDL levels.

Phospholipids in the HDL of starved striped bass remained the same as in the HDL of fed fish on a weight percent basis, but the concentration increased significantly ($P<0.001$). The increased concentration of phospholipids, along with protein, was responsible for the increased HDL in starved striped bass compared with fed. In fact, phospholipids in HDL of starved striped bass accounted for 78% of the total serum phospholipid concentration, whereas phospholipid in fed fish HDL comprised about 50% of total serum levels.

Fish biomembranes contain 25 to 80 percent lipid, mainly phospholipids which are characteristically enriched in polyunsaturated fatty acids (PUFA), primarily 22:6($n-3$) (Henderson and Tocher 1987). The 22:6($n-3$) PUFA is an essential fatty acid in most fish species and is critical to growth and maintaining membrane fluidity in colder environments (Sargent et al. 1989). Striped bass are known to require 22:6($n-3$) for growth and membrane synthesis, and tissue levels of this essential fatty acid were selectively maintained during starvation (Martin et al. 1984). The results of the present study indicate that HDL assumes increased importance in the transport of phospholipids containing essential PUFA for maintenance of tissue integrity during starvation, and perhaps for the synthesis of gonadal tissue during annual reproductive development. The increased role of HDL during reproductive development has been suggested from the results of previous work on salmonids by Nelson and Shore (1974) and Fremont and Marion (1982). High levels of HDL were found in the serum of pink salmon (*Oncorhynchus gorbuscha*) just prior to spawning and after weeks to months of starvation; VLDL and LDL were undetected (Nelson and Shore 1974) but were evident in the early prespawning phase. In this study, phospholipid was about 50% of the lipid in HDL and contained 22:6($n-3$) and 20:5($n-3$) as the predominant PUFAs. Fremont and Marion (1982) found similar results in spermiating rainbow trout, where HDL accounted for greater than 50% of the serum lipoproteins with phospholipid comprising 70% of the lipid in HDL. Additionally, 22:6($n-3$) was the predominant PUFA in the phospholipids. Since phospholipids, enriched in 22:6($n-3$), predominate in egg yolk lipids of striped bass (Eldridge et al. 1983), it is reasonable that HDL functions as a primary vehicle for the transport of structural lipids and essential PUFA for gonadal maturation during the nonfeeding spawning period.

The findings of high serum levels of lipoproteins, and particularly HDL, in striped bass during starvation indicate their important role in permitting fish to withstand long periods without food by distributing lipids to tissues in order to maintain their integrity, whether or not starvation is accompanied by gonadal development. Furthermore, HDL appears to assume greater significance in facilitating the transport of structural lipids and essential PUFA during periods of starvation.

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Contaminated Sediment as a Source of PCBs in a River System

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The transport of PCBs (Aroclor 1242) in a river system in southern Sweden was governed by outflow from sediment in a 26-ha contaminated lake. Experiments in the lake revealed that 14 g PCBs/d escaped from the sediment, while sedimentation was 3 g PCB/d. Volatilization of PCBs from the lake surface was 0.02 g/d, which was considerably higher than the atmospheric fallout (≤0.001 g/d). The majority of the sediment-desorbed compounds (80%) remained at the river mouth, 60 km downstream, and entered the Baltic Sea. Desorption increased the ratio of tetrachlorobiphenyls to pentachlorobiphenyls in the water. Transport across the water/air interface was higher for trichlorobiphenyls, while atmospheric deposition was dominated by penta- and hexachlorobiphenyls. Therefore, the sediment of the lake acted as a source of PCBs entering the river system as well as the atmosphere.

Le transport de BPC (Aroclor 1242) dans un réseau hydrographique du sud de la Suède était régi par l'émission provenant du sédiment d'un lac pollué de 26 ha. Des expériences menées dans le lac ont révélé que 14 g de BPC/d s'échappaient du sédiment, tandis que la sédimentation était de 3 g de BPC/d. La volatilisation des BPC à la surface du lac atteignait 0,02 g/d, ce qui était nettement supérieur aux retombées atmosphériques (≤0,001 g/d). La plus grande partie des composés désorbés par le sédiment (80 %) restait à l'embouchure de la rivière, à 60 km en aval, et entrait dans la mer Baltique. La désorption faisait augmenter le rapport des tétracholorobiphényles aux pentacholorobiphényles dans l'eau. Le transport au travers de l'interface eau/air était plus élevé pour les trichlorobiphényles, tandis que le dépôt atmosphérique était dominé par les penta- et hexachlorobiphényles. Le sédiment du lac constituait donc une source pour les BPC qui entraient tant dans le réseau hydrographique que dans l'atmosphère.

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Some years ago it was discovered that fish from the River Em (catchment area 4460 km^2; mean discharge 30 m^3/s at river mouth (1926–75); maximum estimated 270 m^3/s) in southern Sweden had elevated levels of PCBs. Sediment and fish from a small lake (26 ha, maximum depth 2.5 m, average depth 0.7 m, water residence time about 4 h) situated about 60 km from the river mouth (Fig. 1) showed the highest levels. In the sediment in the lake 14–100 μg PCBs/g dry weight were recorded and northern pike (*Esox lucius*) contained 120–420 μg PCBs/g fat or 0.85–2.2 μg/g fresh weight. Two μg/g fresh weight is the limit above which fish is black listed for human consumption in Sweden. The river is important for recreational fishing of sea trout (*Salmo trutta*) and for sheatfish (*Silurus glanis*) and is known for otter populations (*Lutra lutra*) in its tributaries. A paper mill situated just above the lake was concluded to be the source of the PCBs. The paper mill use recycled paper as raw material, and it was suspected that PCBs in self-copying paper from old archives were the sources of the contaminants. The PCB compounds detected resembled the low-chlorinated, industrial mixture Aroclor 1242.

Studies of PCB transport have been carried out in several contaminated river systems, e.g. in Hudson River (Brown et al. 1985) and Shiawassee River (Rice and White 1987) of the United States and in the River Seine of France (Chevreuil et al. 1987). The objectives of the studies were restricted to follow transport of pollutants in the water, and not to study transport of the pollutants from river water to the atmosphere, sedimentation in the rivers, or atmospheric fallout to the river systems. Additionally, specific PCB congener transport were not followed due to the analytical methods used, information that is valuable when sources of PCBs are to be identified and to determine processes important for the transfer of PCBs between different compartments of the aquatic ecosystem.

The objectives of our study were to determine whether the contaminated sediment in the lake was a source of the PCBs found in the river system and if so, to identify the critical transport processes. Secondly, a mass balance of PCBs for the lake was to be calculated. To carry out these objectives the PCBs present in paper mill discharges were analyzed. The sedimentation of PCBs in the lake was also investigated, as was trans-

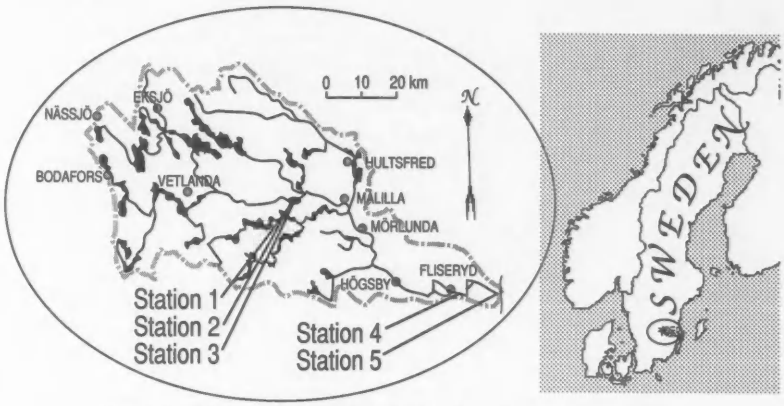

FIG. 1. Location of the sampling stations in River Em, southern Sweden.

port from water to air and atmospheric fallout. To determine the influence of the contaminated lake sediment on PCB transport in the river, PCB-levels in the water were measured down to the river mouth.

Materials and Methods

Sampling

In April 1987, three stations for sampling PCBs in the river water were established: just upstream of the lake (station 2), just downstream (station 3), and 50 km downstream (station 4, Fig. 1). In June, two stations were added, one in the paper mill discharge (station 1) and the other at the river mouth (station 5). At each station about 100 L of river water was filtered through polyurethane filters (PUF, Fischer et al. 1983; Braun et al. 1985; Larsson 1985a) at a rate of 10 mL/min (an electric pump was used, PR G50 Techtum Instruments). Each sampling lasted for a week, after which the filter was exchanged for a new on the same day for all sampling stations. The filters adsorb particulate and dissolved PCBs. PCB transport from sediment to water in the lake was calculated by:

$^{(PCB)}$outflowing water $-$ $^{(PCB)}$inflowing water/area of sediment in the lake·sampling time

In June, 10 undisturbed sediment cores with an overlying water phase were taken from the deepest part of the lake (ca 2.5 m) with a core sampler (Larsson 1983). The cores were transported to the laboratory, placed in a water bath (10°C) and stabilized for a week before starting observations on loss of PCBs from sediment to water. The water phase was then continuously pumped through a PUF (1.4 L/min, Fischer et al. 1983) and returned to the system. The filters were changed weekly. After 28 d the temperature of the water bath was increased to 20°C, and the experiment was continued for another 28 d.

The transport of PCBs from water to air was determined with a sampler described by Larsson and Okla (1987). A defined volume of air (about 12 dm^3) and water surface (11 dm^2) was enclosed by a floating box, opened towards the water surface. Air was passed through two PUF connected in series to remove PCBs was then continuously pumped through the box (at a rate of 3–4 L/min.), and PCB released from the water was then caught on a PUF. Each sampling observation was carried out in duplicate and lasted 1 wk. PCB transport across the water/air interface was calculated as:

($PCB_{\text{total of sampler}}$/sampling time·area of sampling device)·total time of study period·surface area of lake.

The deposition rate of PCBs on the bottom of the lake was measured with two sediment traps modified after Larsson (1984). The trap consisted of two main parts: five collecting sediment vessels (glass tubes closed at one end, 300 mm long and 50 mm in diameter and thus with a height/diameter ratio of 6) and a supporting structure of polyvinylchloride. The traps were moored in the central part of the lake for 3 mo (June–August, 0.3 m above the bottom at a depth of 2.5 m.

The atmospheric fallout of PCBs on the lake was measured with two fallout samplers (Södergren 1972, 1975). The sampler consisted of a metal frame over which a nylon net is stretched. The net is impregnated with a silicone oil, that absorbs lipophilic pollutants and particles. The samplers were placed 3 m above ground at the lake shore and were exposed for 3 mo (June–August).

Sample Preparation

PCBs adsorbed to PUF from the water and air sampling were extracted with acetone/hexane (1:1) in an ultrasonic bath (Larsson 1985b). The hexane fraction containing the PCBs was separated by adding NaCl (2%), evaporated to 1 mL and cleaned-up with several H_2SO_4-additions. PCB adsorbed to particles in the sediment traps were extracted directly in the sediment vessels (Larsson 1984) as described above. PCBs on the fallout samplers were Soxhlet extracted according to Södergren (1972) after which they were cleaned up and evaporated as described above.

PCB Analysis

PCBs in the extracts were analysed by capillary-gas chromatography/ECD. The analyses were carried out with a Varian 3700 GC equipped with a split/splitless injector, and the PCB compounds were separated on a 30-m quartz-capillary column, DB 1 (ID 0.32 mm). N_2 was used as carrier gas (1.2 mL/min) and as make-up gas for the detector (35 mL/min). The GC was programmed in the following mode: initial temperature 60°C in 40 s, directly to 160°C and thereafter 4°C/min to 260°C. The

injector temperature was 250°C, and the detector temperature was 300°C.

As an internal standard, pentachlorobenzene was used. PUF not exposed to water or air contained <5 ng PCBs. Nonexposed fallout samplers contained 49.6 ng (SD = 9.2, $n = 5$), which was subtracted from the amounts obtained on exposed samplers. No PCBs were detected in unused sediment traps. Aroclor 1242 was used as a standard, and the detection limit for this mixture was 0.2 ng. Twenty-one PCB congeners, mainly individual, were quantified using the methods presented by Duinker and Hillebrand (1983) and Ballschmiter and Zell (1980).

Results and Discussion

Inflow of PCBs to the Lake via the River

The highest levels of PCBs in the River Em system were measured in the discharge from the paper mill, >1000 ng/L (Fig. 2). However, because the discharge of water from the mill was low (0.023–0.043 m^3/s) compared with the main river flow to the lake (10–28 m^3/s) the loading of PCBs to the river was marginal. On one occasion, just after the vacation stop in July, the discharge of PCBs from the mill (3 g/d, mean concentration in the water 1094 ng/L, mean discharge 0.037 m^3/s) constituted over 95% of the PCBs flowing into the lake. The PCB compounds in the mill discharge matched the composition of Aroclor 1242. It appears these compounds occasionally contaminate the raw material for the mill when self-copying paper is included in the recycled paper waste.

Transport of PCBs from the Lake Sediment — Field and Laboratory Experiments

Because levels of PCBs in the river water just downstream from the lake were significantly higher than those measured upstream (two-tailed Mann-Whitney U-test, $p<0.001$, Fig. 3), it was concluded that PCBs in the sediment of the lake were released to the water. Levels downstream from the lake varied between 6–36 ng/L whereas in the inflow to the lake they ranged between 0.3–8 ng/L. This corresponds to an outflow of 12.8–47.9 g PCBs/d and an inflow of 0.4–15.7 g/d (Fig. 4). The contribution of PCBs from the sediment was 11.0–42.3 g/d.

The highest levels of PCBs in the outflow of the lake water were inversely related to the water discharge (Fig. 5, linear regression, $p<0.01$). Similar relationships were observed by Brown et al. (1985) in the Hudson River in the United States and by Chevreuil et al. (1987) in the Seine River in France. Therefore, it appears as if the compounds became concentrated during periods of low water turnover, indicating that desorption processes (also known as partitioning processes, Larsson 1983; Wood et al. 1987) had a major influence on the transport.

During periods of high water discharge the water of River Em becomes turbid. However, the release of PCBs from the sediment did not increase during high-flow periods. This would have been the case if the lipophilic and adsorptive PCBs were associated with particles in lake and river water (e.g. Hutzinger et al. 1974). The transport of PCBs across the sediment/water interface did not seem to be affected by particle transport. To explain seasonal changes in PCB levels in fish, it has been proposed that sediments contaminated with PCBs are eroded by spring floods and are taken up by downstream biota (e.g. Olsson et al. 1978). However, it should be pointed out that no extremely high water flows were recorded during this study.

The release of PCBs from the sediment in the lake was not dependent on temperature. The most important processes involved in sediment to water transport are desorption (partitioning), bioturbation (the activity of benthic animals), and gas convection (Södergren and Larsson 1982; Larsson 1983; Karickhoff and Morris 1985; Wood et al. 1987). All these processes are dependent on temperature. Our study in the River Em was carried out during a cold summer when water temperatures fluctuated between 9.8–17.9°C. Only a few of the observations were made when the water temperature was maximum. Therefore, any temperature effect would probably have been difficult to distinguish, especially since discharge varied during the study.

According to our calculations the rate of release of PCBs from the lake sediment (containing 14–100 μg PCBs/g dry weight) to water 1.8–7.7 $\mu g \cdot m^{-2} \cdot h^{-1}$. In the laboratory experiment the transport rate was lower: at 10°C it was 0.027–0.034 $\mu g \cdot m^{-2} \cdot h^{-1}$ (Table 1), increasing to 0.069–0.138 $\mu g \cdot m^{-2} \cdot h^{-1}$ at 20°C (see further below). In a similar laboratory experiment

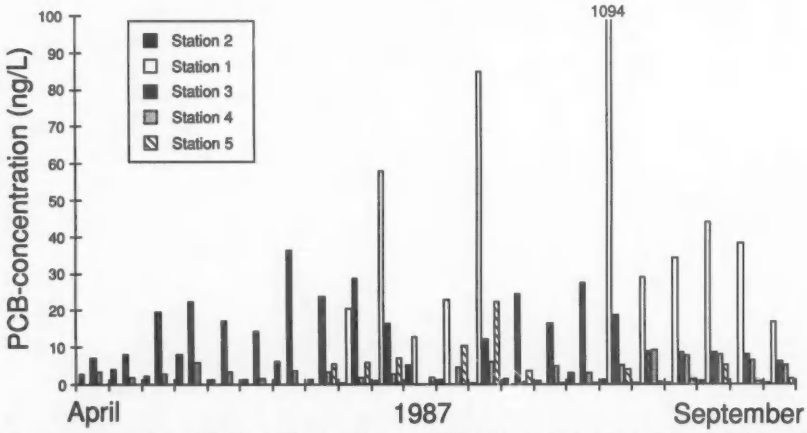

FIG. 2. Concentration of PCBs in River Em water at different sampling stations. PCB sampling was performed continuously from April to September by a filter technique, and the filters were changed weekly.

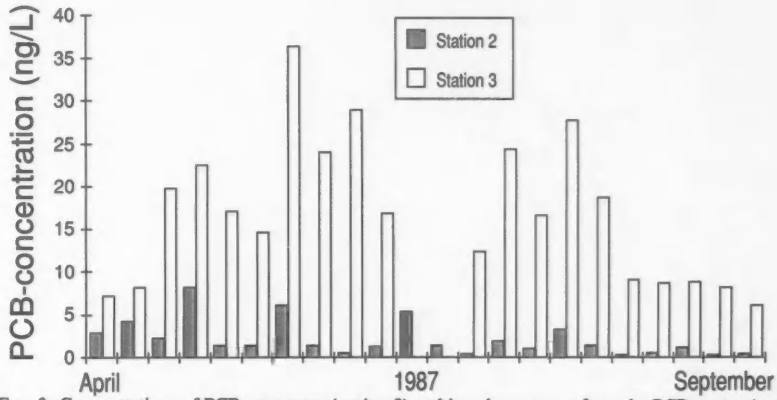

FIG. 3. Concentrations of PCBs upstream (station 2) and just downstream from the PCB contaminated lake (station 3). Two downstream samplings in July are missing owing to a pump failure.

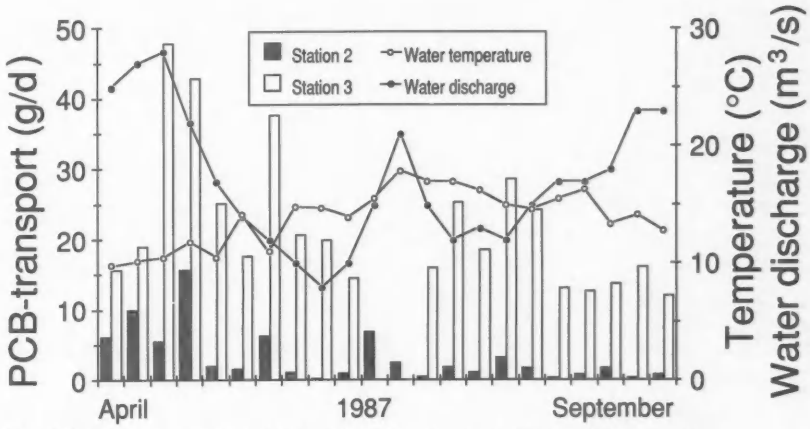

FIG. 4. PCB Transport upstream (station 2) and just downstream (station 3) from the lake.

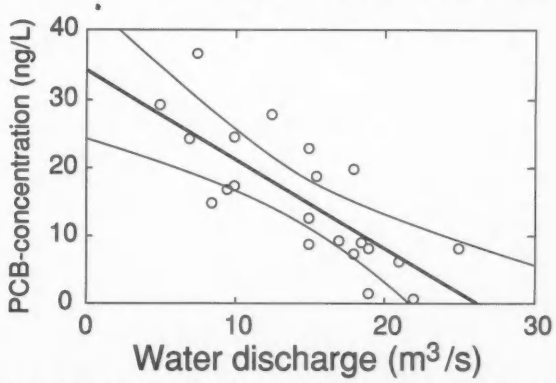

FIG. 5. Relationship ($r^2 = 0.53$, $p = 0.006$) between water discharge and PCB-concentration in the river water just downstream from the lake (station 3). The fine lines denote 95% confidence limits.

(16°C) using specific PCB congeners, Fischer et al. (1983) reported a transport rate of 0.003–0.019 $\mu g \cdot m^{-2} \cdot h^{-1}$ (the PCB concentration in the sediment was 0.17–1.65 $\mu g \cdot g^{-1}$ dry weight). In a static laboratory experiment Halter and Johnson

TABLE 1. Laboratory studies of the transport of PCBs across the sediment/water interface. Ten sediment cores with an overlying water phase were used in the study. Numbers within brackets refer to standard deviation.

Incubation time (d)	Transport rate ($\mu g.m^2.\ h_1$) 10°C	20°C
7	0.027 (0.011)	0.069 (0.032)
14	0.028 (0.015)	0.093 (0.033)
21	0.034 (0.017)	0.107 (0.060)
28	0.030 (0.012)	0.137 (0.222)

(1977) found a transport rate of 0.006 μg Aroclor 1254·m^{-2}·h^{-1} (at a concentration of 200 μg/g in the sediment). In large mesocosm studies in the field, Clophen A 50 (comparable with Aroclor 1254) was released from the sediment (containing 3.2 μg PCB/g dry weight) at a rate of 0.008–0.071 $\mu g \cdot m^{-2} \cdot h^{-1}$ (Larsson and Södergren 1987). The systems were of continuous-flow type, and transport was, among other factors, dependent on the water temperature and on the influence of benthic macroinvertebrates and fish. The comparison between the River Em and the mesocosm study seems to be the most relevant, since the latter study was carried out in the field

under continuous-flow conditions. The higher transport rate observed in the river was probably due to the higher concentrations of PCBs in the sediment, which combined with the turbulence in the river bottom increased the active surface of the sediment exposed to the water leading to increased desorption (Fischer et al. 1983; Wood et al. 1987). In addition, turbulence probably brought contaminated material deeper in the sediment to the sediment/water interface.

The organic content of the sediment is of crucial importance in determining the extent of PCB transport across the sediment/water interface (Steen et al. 1978; Karickhoff et al. 1979). Sediments rich in organic compounds adsorbs the lipophilic PCB compounds to a higher degree than minerogenic sediments. The organic content of the lake sediment was 13–20% of its dry weight (measured as the ignition loss at 530°C).

In the laboratory study a winter (10°C) and a summer (20°C) situation were simulated (Table 1). At the higher temperature, the release rate increased by a factor of two to four. As no macroinvertebrates were present in the laboratory sediment, desorption dominated the transport. We were able to simulate conditions prevailing in the lake by using a sampling technique allowing the sediment stratification to be maintained in the cores (Södergren and Larsson 1982; Larsson 1983) and by keeping the surface layer of the sediment intact. In a laboratory experiment using spiked Lake Ontario sediment, Oliver (1987) found that PCB congener transport increased three to seven-fold when the temperature was raised from 8°C to 20°C. The highest desorption rate was recorded for PCB 52, which is consistent with the findings in our study. Release rates of PCB congeners were higher in the study performed by Oliver than in ours, probably due to bioturbation activity in the sediment caused by oligochaete worms.

PCB desorption from sediment follows a first-order rate of kinetics, and once the asymptote is reached (at about 30 d, Wood et al. 1987) transport decreases (Fischer et al. 1983; Wood et al. 1987). In the field, desorption is maintained by disturbance of surface sediment caused by water movement. PCB congeners will partition from sediment to water at a rate determined by the inverse of their octanol/water partition coefficient (roughly inverse to their lipophilicity, Wood et al. 1987). In our experiments at 20°C the microbial activity in the sediment began to increase, and after about 7 d gas bubbles started to develop in the sediment. The gas convection increased turbidity in the overlying water phase, probably mediating PCB transport by particles across the sediment/water interface (Södergren and Larsson 1982; Larsson 1983). The phenomenon did not occur in all the cores; thus variation in PCB transport was higher on the last sampling observation (Table 1). During the field study 20°C was not reached in the River Em because the summer was unusually cold. However, if a water temperature of 20–25°C had been reached as is usual, microbial activity would have increased, probably resulting in more transport of PCBs from the lake sediment to the river.

Influence of Contaminated Lake Sediment Downstream and at the River Mouth

The PCB concentrations in the river water at the localities 50 km downstream (2.1–8.2 ng/L) and at the river mouth (0.5–22.5 ng/L) were lower than those recorded at the outflow of the contaminated lake. These concentrations are equivalent to a transport of 4–28 g PCBs/d at station No. 4 and 1–58 g/d at the river mouth (station No. 5, Fig. 6). Calculations of PCB transport were more uncertain at these two sampling stations than at the station at the lake outlet owing to considerably higher and variable discharge (26–68 m^3). Contaminated lake sediment accounted for a substantial part of the PCB transport and affected the whole river system; i.e. amounts of PCBs measured at the river mouth were 60–70% of those measured at the lake outlet. The majority of the PCBs released from the sediment (0.3–0.4 kg/mo) reached the Baltic Sea.

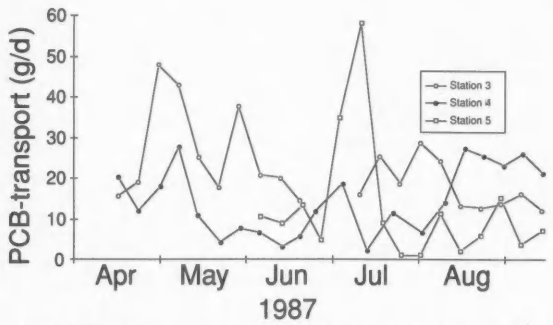

FIG. 6. PCB transport at station 3, station 4, and at the river mouth (station 5).

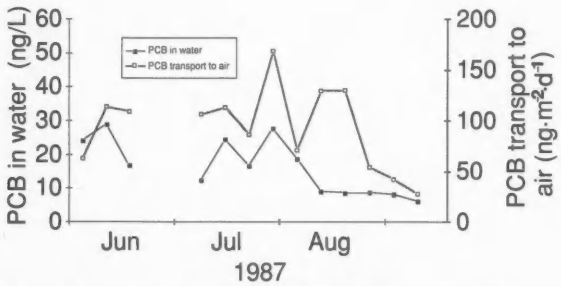

FIG. 7. Concentration of PCBs in the water just downstream from the lake (station 3) and PCB transport to air. PCB transport to air was measured with duplicate samplers.

PCB Transport from Water to Air

PCBs in the lake water were volatilized to the atmosphere (Fig. 7). The volatilization rate was 1.2–7.0 $ng \cdot m^{-2} \cdot h^{-1}$, which is comparable with the rates recorded in mesocosm studies in the field (0.9–9.6 $ng \cdot m^{-2} \cdot h^{-1}$) using the same sampling equipment (Larsson and Okla 1987). The transport probably is governed by Henry's Law (Liss and Slater 1974; Bopp 1983) and roughly determined by the concentration of PCBs in the water and water temperature. However, no consistent relation between the concentrations of PCBs in the lake water and the transport rate to air was found (Fig. 7). Although such a relationship existed between June and the end of July, it was not found during August and September. It is the dissolved PCB fraction that is available for volatilization across the water/air interface, and this fraction probably varied during the sampling period. Also, levels of dissolved organic carbon (humic substances) which are known to adsorb PCBs (Chiou et al. 1986) varied in the river water over the season. We calculated that the movement of PCBs from the lake water to the atmosphere was 2.5 g from June to September. This value is probably lower than usual because of cooler than average water temperatures (≤18°C).

PCB transport is affected by wind, wave induction, and turbulence in the water. Moreover, the sampling technique should have excluded these effects.

Transport of Individual PCB Congeners — Pattern Recognition

In general, the PCB pattern observed in the contaminated lake resembled that of Aroclor 1242. However, owing to differences in their chemical properties, specific congeners behave differently when desorbed from the sediment or volatilized to air. The water solubilities of several PCB congeners have been experimentally determined by Murphy et al. (1987). In principal, the water solutility of the congeners should decrease as the number of chlorine substituents increase. When desorbed from sediment in the laboratory studies as well as in the water leaving the lake, the congeners which increased their relative distributions were the trichlorobiphenyls 28 (2,4,4′) and 31 (2,4′,5), with a water solubility of 143 μg/L, and the tetrachlorobiphenyls (in decreasing order): PCB 52 > 44 > 41 > 49 (Fig. 8, IUPAC number, Ballschmiter and Zell 1980). The water solubility of PCB 52 is 112 μg/L; PCB 44, 100 μg/L; PCB 41, 65 μg/L; and PCB 49, 78 μg/L (Murphy et al. 1987). Consequently, it seems as though the solubility of the congeners determined their degree of desorption across the sediment/water interface. It should be pointed out that the chemical properties of some PCB congeners in this investigation have not been reported in the literature and that the PCB congeners that could not be quantified individually (co-eluting) were withdrawn from the comparison (70 + 80 + 98, 95 + 66, 47 + 75). The greatest divergences from the composition of congeners in the Aroclor 1242 mixture were observed for PCB 18 (2,2′, 5-triCB), which

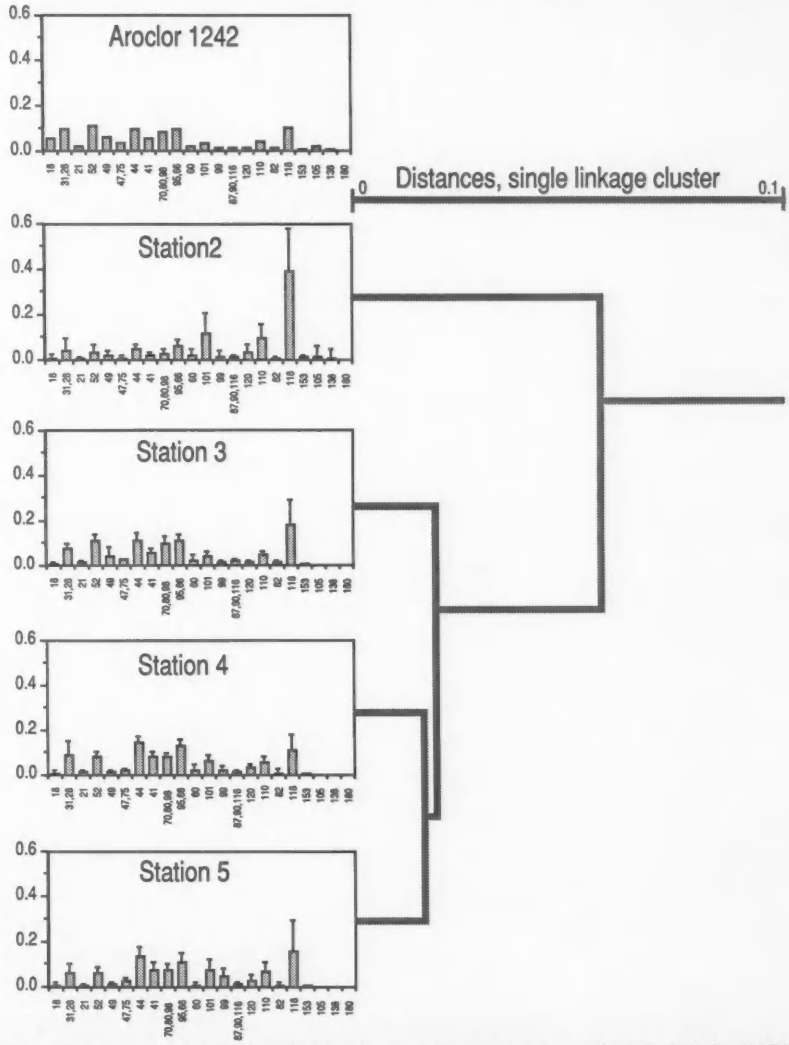

FIG. 8. PCB congener distribution (fraction of total PCBs, mean of all samplings) in the industrial PCB mixture Aroclor 1242, in the water of station 2, station 3, and at station 5. The degree of similarity in congener distribution between the stations was tested by cluster analysis (Hopke et al. 1976).

was desorbed from sediment to a much lesser extent, and for PCB 118, (2,3′,4,4′,5) which was present in higher proportions in the water. PCB 18, which is a major component of Aroclor 1242 (5.7%), has been shown to occur less frequently in sediments contaminated with this PCB-mixture (Brown et al. 1987). This difference could be due to microbial degradation, as suggested by the fact that this congener is broken down by bacterial strains isolated from PCB-contaminated sediment from the Hudson River in the United States (Bedard et al. 1986). The major metabolites formed during the degradation of biphenyls with the substitutions 2,2′,5-, 2,5,3′-, and 3,4,2′- are dichlorobenzoic acids (Furukawa et al. 1979). PCB 118 was the congener whose total distribution decreased the most (21%) between the inlet and the outlet of the lake. This decrease was probably due to sedimentation.

The partitioning coefficients between dissolved and particulate phases of PCBs have been investigated in Lake Superior (Baker et al. 1986). Of the congeners common to both the River Em and Lake Superior the trichlorobiphenyl PCB 31 was most abundant in the dissolved state. The dissolved/particulate quotients of the tetrachlorobiphenyls in Lake Superior decreased in the order PCB 41>52>49>44. This was quite different compared with their ranking with regard to desorption from the lake sediment in River Em, which was PCB 44>52>41>49. This difference between lakes could have been due to the different PCB sources, to differences in the proportions of congeners in the Arochlor mixtures discharged as well as to different particle properties. In Lake Superior the pentachlorobiphenyls partitioned to a higher extent to particles than did the tetrachlorobiphenyls. There was one exception, however, namely PCB 118, wich also was a major component in the water of the River Em. One surprising finding by Baker et al. (1986) was that the observed sediment–water distribution of PCBs was independent of the properties of highly hydrophobic compounds, i.e. the octanol/water partitioning coefficients (lipophilicity or roughly the reciprocal water solubility) did not accurately predict the PCB speciation between seston and water.

In an excellent laboratory study, Wood et al. (1987) investigated the desorption of PCB congeners from a sediment spiked with a mixture of Aroclor (1016, 1221, 1254, and 1260). Their conclusions were than the congeners desorbed from sediment to water at a rate that was inversely related to their octanol/water partitioning coefficients (increasing lipophilicity). In principle, this means that an increase in the number of chlorine substituent on the biphenyl resulted in a lower release rate. Unfortunately, Aroclor 1242 was not used in the study, which made a comparison with our study impossible because the congeners in nearly all cases were different.

As the transport of PCBs was higher at the lake outlet than at the inlet, transport of all congeners in Aroclor 1242 increased. However, when comparing the relative congener distribution of PCBs in water entering the lake (Fig. 8) with the distribution of PCBs redistributed from the sediment in the river, results similar to those of Wood et al. (1987) were obtained. There were increases in the proportion of all tri- and tetrachlorobiphenyls as a result of sediment redistribution, whereas nearly all pentachlorobiphenyls decreased. The two hexachlorobiphenyls present in the water at the lake inlet were not detected in any of the samples at the outlet.

The congener distribution did not show any major changes between station No. 3, station No. 4, or No. 5. The distributions of the PCB congeners in the water upstream from the lake (station No. 2), No. 3, No. 4, and at station No. 5 were compared using cluster analysis (Fig. 8). Samples taken downstream from the lake all showed great similarity in their PCB pattern, whereas the PCB pattern in the water from the station upstream from the lake was dissimilar from the rest. The results indicate that PCB compounds at the river mouth originated from the lake sediment. Based on the assumption that no new addition of PCBs occurred during the transport, it can be concluded that the relative amounts of PCB 52 (tetra CB) and PCB 49 (tetra CB) decreased during transport while those of PCB 41 (tetra CB), PCB 44 (tetra CB), and PCB 101 (pentachloro CB) increased.

When the PCB congeners were volatilized from the lake water across the water/air interface, transport of the trichlorobiphenyls PCB 28 and PCB 31 was higher compared with the other congeners measured. Their combined relative distribution in air was 19.6% compared with 7.5% in water. The vapour pressure of these two trichlorobiphenyls is $147 \cdot 10^{-9}$ atm, which is considerably higher than for any other congener of Aroclor 1242 (Murphy et al. 1987). Relative amounts of all trichlorobiphenyls increased when volatilized, probably because they have fewer chlorine substituents that tetra- and pentachlorobiphenyls, thereby giving them a higher volatility. The relative proportion of PCB 44 (tetra CB) also increased when transported to air. This result cannot be explained solely on the basis of vapor pressure differences, since PCB 49 and 52 have higher vapor pressures ($63 \cdot 10^{-9}$ atm versus $73 \cdot 10^{-9}$ atm, and $89 \cdot 10^{-9}$ atm, respectively). In principal, vapor pressure decreases as the number of chlorine substituents increases.

Mass Balance and Uptake by Biota

Since the lake is fairly small with a defined inflow and outflow and no other sources of PCBs, it was possible to calculate a mass-balance with reasonable accuracy (Fig. 9), specifically on July 20. In July, 2.0 g PCBs/d entered the lake with inflowing water of which PCB 118 constituted 0.9 g and PCB 101, 0.4 g. The release from the sediment was at least 14 g/d. The transport across the sediment/water interface was dominated by five congeners, PCB 118 (2.3 g), PCB 52 (1.7 g), PCB 70 + 80 + 98 (1.6 g), PCB 95 + 66 (1.6 g), and PCB 44 (1.5 g). Sedimentation of PCBs was 3 g/d. However, this was probably not a result of a net transport to the bottom; the high accumulation rate in the traps indicated that the bottom sediment was resuspended. This conclusion was further supported by the fact that sedimentation of PCB congeners was different from that in the inflowing water; 10 congeners constituted each between 0.13–0.35 g/d (Fig. 9).

The precision of the sediment traps was high. The first trap collected 54.1 g dry material$\cdot m^{-2} \cdot d^{-1}$ (mean of two observations; a loss of ignition 31%) resulting in a sedimentation rate of 12.5 μg PCB$\cdot m^{-2} \cdot d^{-1}$ (SD = 8.5, $n = 3$). The second trap collected 52.8 g dry material$\cdot m^{-2} \cdot d^{-1}$ (51.8–53.9 g, with a loss of ignition of 33%) with a PCB-sedimentation rate of 7.9 μg PCB$\cdot m^{-2} \cdot d^{-1}$ (SD = 1.9, $n = 3$). This sedimentation rate was similar to that of dimictic lakes known to be subjected to resuspension during spring and autumn overturn (Larsson 1984). In these lakes gross sedimentation reached 93 g dry matter$\cdot m^{-2} \cdot d^{-1}$, rates higher but similar in magnitude to those obtained in the lake on the River Em. Because of the characteristics of the lake (shallow, with the River Em main flowing through) resuspension of bottom sediments was considered highly probable. On the other hand, the fact that the levels of PCBs were much lower in sedimenting material (0.22 μg/g dry

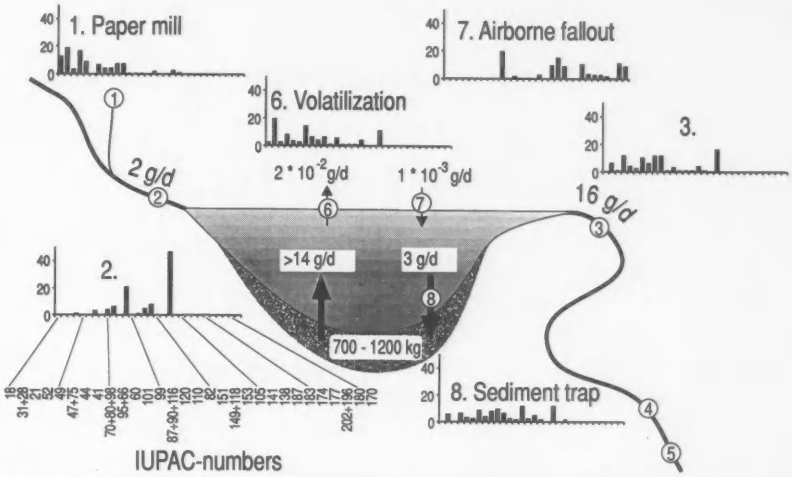

FIG. 9. Mass-balance of PCBs in the contaminated lake on July 20, 1987. PCB congener distribution (IUPAC numbers, percent) for the different flows (g/d) is shown above each main process. Note that the congener distribution of PCB transport from sediment to water is the same as just downstream lake. The theoretical retention time of water was about 4 h.

weight, SD = 0.17, $n = 6$) than in the surface sediment suggests that transport of incoming, low-contaminated material to the lake was substantial. This material probably gets mixed into the sediment during flooding events, rather than acting as a barrier to PCBs release at the sediment surface (Larsson 1985a; Larsson and Södergren 1987). During the study, the release rate of PCBs from the sediment exceeded the sedimentation rate.

In July, the volatilization rate of PCBs was $2 \cdot 10^{-2}$ g/d, which was about 10 times the atmospheric fallout rate ($1 \cdot 10^{-3}$ g/d). Volatilization was highest for PCB 28 + 31 that constituted 19.6% of the PCB congeners trapped in the sampler. The next most frequent congeners were PCB 44 (14.2%), PCB 118 (11.6%), PCB 52 (8.6%), and PCB 95 (7.0%).

The atmospheric fallout of PCBs was 4.50 and 5.27 $ng \cdot m^{-2} \cdot d^{-1}$. Unlike any other abiotic component of the lake ecosystem, the PCB pattern atmospheric deposition resembled a highly-chlorinated industrial PCB mixture, Clophen A 60. This deposition pattern is generally found throughout Sweden (Larsson and Okla 1990). Dominating congeners in atmospheric deposition were PCB 95 (19.4%)>PCB 149 + 118 (14.9%)>PCB 180 (11.2%)>PCB 138 (10.4%)>PCB 151 9.6%. The results point out that the PCBs volatilized from the lake water did not immediately return as atmospheric deposition. Volatilization constituted <0.1% of the total PCBs in transport, which was lower than the 0.3% found in a mesocosm experiment (Larsson and Södergren 1987). The volatilization process probably is of great significance for the long-range transport of persistent pollutants like PCBs; in their gaseous state the compounds can be transported by wind over long distances, thereby contributing to the global transport. On the other hand, PCB transport in water seems to be of local significance only — at least in the short term. The contaminated sediment of the lake was a significant source of PCBs entering the river system but was relatively unimportant quantitatively as a source to the atmosphere in the immediate vicinity of the river Em.

The contaminated sediment of the lake was the most important factor for PCB transport in the River Em from April to September 1987. Over 80% (2.8 kg) of the maximum PCB load measured in the river (3.3 kg) can be attributed to this source. The paper mill burdened the river with low amounts of PCBs (0.03 kg) during the study period; however, movement to the river probably occurred irregularly, during short periods when the raw material was contaminated. Fifty kilometres downstream from the lake at station No. 4, amounts of transported PCBs were less (2.2 kg). This decrease was probably due to adsorption to the sediment, volatilization losses, and uptake by biota. At the river mouth, total transport was 1.4 kg. However, because this calculation was based on a 6-wk shorter sampling period compared with the upstream stations actual transport must have been higher.

The highest concentrations of PCBs in the contaminated lake could theoretically result in a level exceeding 2 mg/kg (fresh weight) in fish, based on uptake in fish by equilibrium partitioning (Brown et al. 1985). These levels were found in pike in the contaminated lake. Direct uptake from water is fast, a matter of hours to days (Pizza and O'Connor 1983). Further downstream, the uptake by partitioning in fish is reduced, probably due to greater dilution of the PCB compounds. Organisms in the River Em can also obtain PCBs via their food. A steady state is reached more slowly as a result of uptake via food, and tissue PCB levels increase with age (Spigarelli et al. 1983). Thus the hazardous buildup of PCB in the river biota occurs in two main ways: one characterized by a fast, direct uptake of pollutants in the most contaminated areas and the other characterized by a slower uptake via food in highly contaminated areas as well as in areas downstream.

International Comparisons with Other Rivers

By international standards, the contamination of the River Em is of low magnitude. The Hudson River (United States) was contaminated by the PCB mixtures Aroclor 1016 and 1242 originating from two [illegible] former factories. The sediment of the river contained [illegible] PCBs/g in many locations, and even exceeded 1000 [illegible] in some areas (Brown et al. 1985). Fish had levels of 10–[illegible]30 μg/g (fresh weight), depending on species

and location. Concentrations of PCBs in the water were ca 1 μg/L during periods of low river flow (100 m^3/s) and ca 0.2 μg/L during intermediate flow (400–500 m^3/s), resulting in a transport of 900 g PCBs/d at the intermediate flow rate. In the Shiawassee River, a smaller river in the United States with contaminated sediment, (21–533 μg/g) concentrations in the water were 0.65–1.10 μg/L before the sediment was removed (Rice and White 1987). During restoration, levels of PCBs in the water increased to between 1.03 and 4.67 μg/L, and later decreased to 0.52–1.11 μg/L. PCBs released to the water were taken up by mussels (13–69 μg/g dry weight) and fish (32–88 μg/g dry weight).

In the River Seine in France, PCB concentrations in sediment were comparable with those measured in the two United States studies (Chevreuil et al. 1987). Concentrations of PCBs in the river water varied between 13–120 ng/L depending on location. One conclusion of the authors was that PCBs were still being discharged into the river from unknown sources.

In conclusion, the level of contamination of the three abovementioned river systems was greater than that recorded in River Em. However, these three rivers should be seen as extreme examples of pollution, and the risks posed by PCBs in River Em should not be underestimated. The contaminated lake sediment which was less polluted than the sediment in the rivers in the United States and France acted as a source of PCBs entering the river as well as the atmosphere.

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Effects of Extended Daylength on Autumn Growth Dynamics of Juvenile Atlantic Salmon, *Salmo salar*

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The growth and growth dynamics of juvenile Atlantic salmon (*Salmo salar*) exposed from mid-August to mid-December to either a normal light:dark cycle (LDN) or a constant 16-h light:8-h dark (LD 16:8) photoperiod were compared. Exposure to the LD 16:8 resulted in 38.1% of the fish entering the upper modal group (UMG) of the length–frequency distribution, while only 15.4% entered the UMG under LDN. The length at which fish entered the UMG (critical fork length) did not differ between photoperiods. Fish in the small (5.8–6.7 cm) and middle (6.8–7.7 cm) size-ranges under LD 16:8 had greater growth rates than fish in the same size-ranges under LDN. Fish in the large (8.3–9.2 cm) size-range showed no significant variation in growth rates between photoperiods. At the end of the experiment, mean thyroxine (T_4) levels were significantly ($P<0.05$) lower in fish from the LD 16:8 photoperiod (1.69 ng/mL) in comparison with LDN fish (3.96 ng/mL). Triiodothyronine (T_3) levels and the T_3/T_4 ratio were elevated under LD 16:8.

Nous avons comparé la croissance et la dynamique de croissance chez des jeunes saumons de l'Atlantique (*Salmo salar*) soumis de la mi-août à la mi-décembre à deux régimes d'éclairement : cycle normal lumière/obscurité (LDN) ou photopériode constante de 16 h de lumière et de 8 h d'obscurité (LD 16 :8). Avec l'exposition au régime LD 16 :8, 38,1 % des poissons sont entrés dans le groupe modal supérieur (UMG) de la distribution longueur-fréquence, alors que cette proportion n'était que 15,4 % dans le régime LDN. La longueur à laquelle les poissons entraient dans l'UMG (longueur à la fourche critique) ne variait pas d'un régime à l'autre. Les poissons des catégories de taille petite (5,8–6,7 cm) et moyenne (6,8–7,7 cm) sous le régime LD 16 :8 présentaient des taux de croissance supérieurs à ceux des poissons des mêmes catégories sous le régime LDN. Les poissons de la catégorie de grande taille (8,3–9,2 cm) ne présentaient aucune variation notable dans le taux de croissance d'un régime à l'autre. A la fin de l'expérience, le taux moyen de thyroxine (T_4) était nettement plus bas ($P<0,05$) chez les poissons soumis au régime LD 16 :8 (1,69 ng/mL) que chez les spécimens soumis au régime LDN (3,96 ng/mL). Le taux de triiodothyronine (T_3) et le rapport T_3/T_4 étaient élevés sous le régime LD 16 :8.

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Juvenile Atlantic salmon (*Salmo salar*) raised under hatchery conditions develop a bimodal distribution in length during their first year of growth (Simpson and Thorpe 1976; Thorpe 1977; Bailey et al. 1980). Segregation into this bimodal distribution begins to occur in late August when the larger fish in the population enter a short-lived period of rapid growth to form the upper modal group (UMG); at the same time, the smaller fish undergo a decrease in growth rate to form the lower modal group (LMG) (Kristinsson et al. 1985; Metcalfe et al. 1988). Development of bimodality is due to an increase in appetite in UMG fish, whereas the appetite of LMG fish declines (Higgins and Talbot 1985; Metcalfe et al. 1988). Although the growth rates of the UMG fish subsequently decline after this growth spurt, fish maintain a higher growth rate throughout the winter compared with fish in the LMG (Thorpe 1977; Kristinsson et al. 1985; Higgins and Talbot 1985). Individuals in the UMG complete smoltification the following spring, whereas those in the LMG require an additional year in fresh water before smoltifying (Simpson and Thorpe 1976; Thorpe 1977; Thorpe and Morgan 1980).

Photoperiod plays an important role in the development of bimodality in juvenile Atlantic salmon. Six- and nine-month phase delayed photoperiods delay the development of the bimodal distribution and decrease the percentage of fish entering the UMG (Thorpe 1986; Villarreal et al. 1988). In contrast, an abrupt increase from ambient (approximately 13.5 h light:10.5 h dark) to a LD 16:8 daylength in late August increases the number of fish in the upper modal group (Saunders et al. 1990). The objective of this paper is to determine the processs through which an abrupt increase in daylength in autumn alters fish growth and increases the proportion of fish entering the UMG. Extended daylength could decrease the critical fork length a fish must reach to enter the UMG or increase growth rate, thus allowing more fish to reach the critical fork length and enter the UMG. In order to ascertain which of the above factors is responsible for enhanced recruitment into the UMG, we have utilized individually marked salmon parr. Thyroxine (T_4) and triiodothyronine (T_3) were measured to determine if extended daylength had any effect on thyroid status. Previous studies have reported elevated T_4 levels in fish from the UMG (Simpson and Thorpe 1986; Kristinsson et al. 1985).

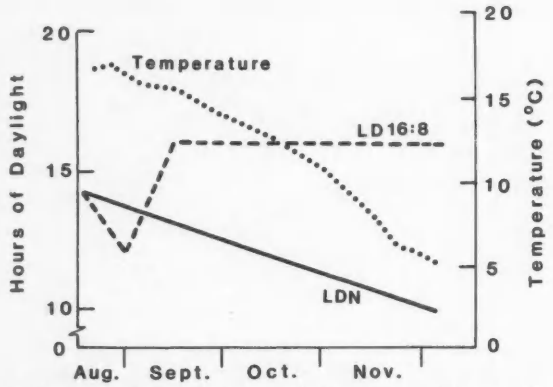

FIG. 1. Photoperiods and mean weekly temperatures (°C) during the experimental period.

Materials and Methods

Two hundred and fifty Atlantic salmon parr (Saint John River, NB stock) of mixed parentage were individually heat branded in mid-August. The fish were randomly separated into two groups with one group being placed on a normal light:dark cycle (LDN). The second group was placed on a photoperiod which initially decreased from 14 h daylight on August 15 to 12 h daylight on September 1 and then gradually increased to a constant 16 h light:8 h dark (LD 16:8) by September 15 (Fig. 1). The initial reduction in the LD 16:8 photoperiod was included to exaggerate the final increase to 16 h light. It was believed that a direct increase to 16 h daylength from the natural photoperiod daylength of 14 h at the start of the experiment would be insufficient to stimulate growth. Standard fluorescent bulbs were used and provided light intensities of 70–120 lux at the water surface.

The fish were held in 100 × 100 × 55-cm semi-square (Swedish type) fiberglass tanks. Dechlorinated fresh water was supplied to the tanks at a rate of 17.5 L/min. Water temperatures declined from 17.6°C at the start of the experiment to 4.7°C at its termination (Fig. 1). The fish were fed an excess ration of commercial dry salmon food (Corey Feed Mills) at 1/2-h intervals throughout the LDN daylight hours.

On September 15, all fish were re-marked since the brands were fading. Fish from both photoperiods were treated with brackish water (15/20 ‰) on September 20 to counter fungal infections. A drain malfunction in late September in the tank housing the LDN group resulted in mortalities ($n = 38$). The fish that were lost from the experiment were not significantly different in length ($P > 0.95$) from surviving fish based on lengths from the previous measuring period. Fish that died were not included in the data analysis.

On December 10, plasma samples were taken from fish in the UMG. Plasma was stored at −80° until thyroxine (T_4) and triiodothyronine (T_3) levels were simultaneously assayed by D. Cyr, Department of Zoology, University of Manitoba, Canada. The method of Omeljaniuk et al. (1984), as modified by Cyr et al. (1988), was used.

Data Analysis

Fish were measured to the nearest 1 mm at 2-wk intervals for the first 2 mo and monthly thereafter for a total of six measurement intervals. Differences in mean length between the respective modal groups at the end of the experiment were compared between photoperiods using a t-test with a $P < 0.05$ level considered significant.

Linear growth rate (LGR) for each individual was calculated as:

$$\text{LGR (mm/d)} = (FL_2 - FL_1) / (T_2 - T_1)$$

where FL_2 and FL_1 are fork lengths at times T_2 and T_1, respectively. This method of determining growth rate was used in lieu of instantaneous growth rate based on arguments presented by Kristinsson (1984). One-way ANOVA and SNK were used to test for significant changes in linear growth rate over time within a photoperiod treatment. Student's t-test was used to compare mean LGR between photoperiods at any given time. Two-way ANOVA was used to analyze LGR profiles to determine if a significant interaction between photoperiod and time existed.

The differential growth rate of fish from the UMG and LMG is apparent at an earlier date in the linear growth rate versus fork length (LGR versus FL) scatter diagrams than in the plots of length–frequency distributions (Kristinsson 1984). Therefore, plots of LGR versus FL for the final measurement were used in modal determinations. The proportion of fish in the UMG for the LDN and LD 16:8 photoperiods was compared using the normal approximation to the binomial (Brownlee 1960).

For each measuring date, the fork length at which fish entered the UMG was determined by drawing a borderline at a break in the distribution of the LGR versus FL scatter diagrams such that all fish to the right had permanently entered the stage of faster growth associated with the UMG (see Kristinsson 1984). The length at which an individual crossed this borderline was determined to be the critical fork length (CFL). Differences in critical fork length were compared using a t-test.

Differences in plasma T_4 and T_3 levels and T_3/T_4 ratio were compared using a t-test for unequal variances.

Results

At the end of the experiment, the linear growth rate versus fork length diagrams indicated that the length distribution was bimodal (Fig. 2) with a break in the distribution at 12.5 cm. Exposure to the LD 16:8 photoperiod resulted in 38.1% of the fish entering the UMG, which was significantly ($P < 0.01$) greater than LDN in which only 15.4% of the fish entered the UMG. Mean fork lengths of fish in the respective upper and lower modal groups did not differ between the two photoperiod treatments.

The mean critical fork length of fish exposed to the two photoperiods did not differ (LDN = 10.64 cm; LD 16:8 = 10.75 cm). The short-lived period of rapid growth associated with entrance into the UMG occurred between early September and mid-October for fish exposed to LDN. Entrance into the UMG for the LD 16:8 fish took place from the beginning of September to mid-November. There was a significant positive correlation between critical fork length and the time of entry into the UMG for both photoperiods (LDN $r^2 = 0.78$, $P < 0.01$; LD 16:8 $r^2 = 0.69$, $P < 0.001$).

Under LDN, fish in the 6.8–7.2 and 7.3–7.7 cm size-classes in mid-August did not enter the UMG (Fig. 3). In contrast, exposure to the LD 16:8 photoperiod allowed 15 and 39% of fish in these respective size-classes to enter into the UMG. Compared to LDN, exposure to LD 16:8 also enhanced the

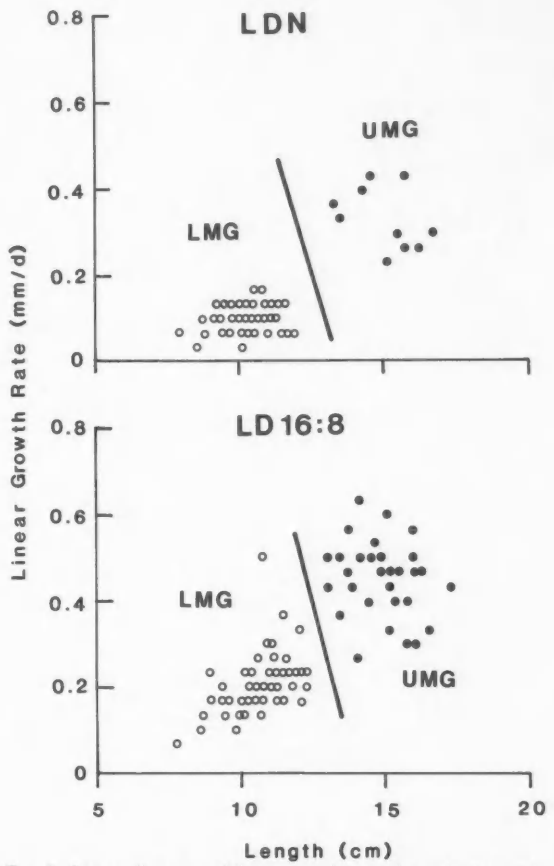

FIG. 2. Scatter diagrams of linear growth rate (determined over the previous 30 d) versus fork length determined at the end of the experiment for fish under the LDN and LD 16:8 photoperiods. The break in the distribution at 12.5 cm separated the upper and lower modal groups for each photoperiod. Symbols may represent more than one fish due to overlap.

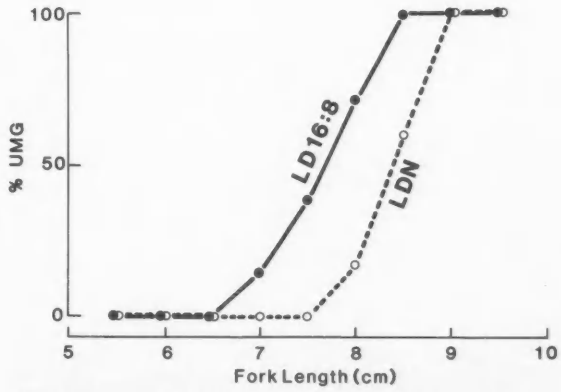

FIG. 3. Percentage of fish in a given size-class at the start of the experiment which entered the upper modal group at the end of the experiment. The symbols are plotted on the midpoint of the size-class.

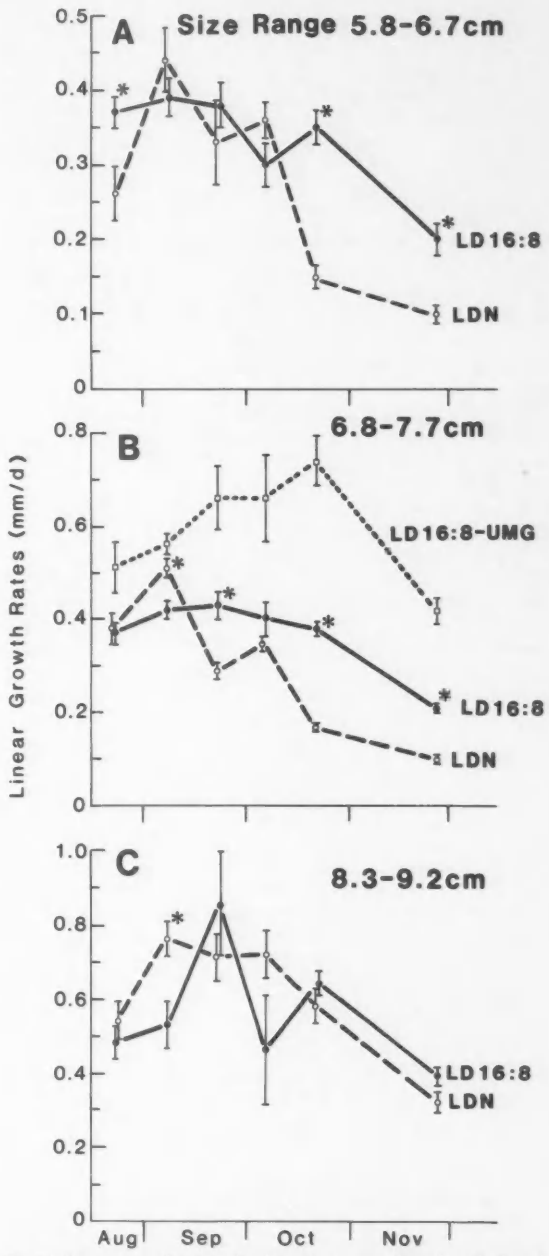

FIG. 4. Linear growth rates of various size-ranges (based on the length at the start of the experiment) during the course of the experiment. Growth rates are plotted in the middle of the measurement interval. Fish in the 5.8–6.7 cm size-range were in the LMG at the end of the experiment. For the 6.8–7.7 cm size-range, growth rates for fish which remained in the lower modal group are represented. Growth rates of fish in this size-range which entered the UMG under the LD 16:8 photoperiod are presented for comparative purposes (L16-UMG) and are not included in the statistical analysis. Fish in the 8.3–9.2 cm size-range entered the UMG by the end of the experiment. * indicates a significant difference ($p<0.01$) in growth rates between the two photoperiods at a given time.

TABLE 1. Mean plasma thyroxine (T_4), triiodothyronine (T_3) and T_3/T_4 levels (SE) in upper mode fish (>12.5 cm) from the LDN and LD 16:8 photoperiods at the termination of the study.

Photoperiod	n	T_4 (ng/mL)	T_3 (ng/mL)	T_3/T_4
LDN	7	3.96 (0.84)[a]	1.90 (0.48)	0.52 (0.08)[b]
LD	9	1.69 (0.24)[a]	2.41 (0.39)	1.68 (0.31)[b]

[a]$p<0.05$.
[b]$p<0.01$.

proportion of fish reaching the UMG from the 7.8–8.2 and 8.3–8.7 cm size-classes.

Based on the fork length at the beginning of the experiment, mean linear growth rates were determined for three size-ranges of fish for each respective photoperiod. The small size-range (5.8–6.7 cm) was chosen since none of these fish entered the UMG. The middle size-range (6.8–7.7 cm) represents transition fish. Although fish in this size-range do not enter the UMG under LDN, exposure to LD 16:8 fish results in some fish entering the UMG. The large size-range (8.8–9.7 cm) under both LDN and LD 16:8 represented, without exception, fish which entered the UMG by the end of the experiment.

LD 16:8 small size-range fish had significantly greater ($P<0.01$) growth rates than its counterparts under LDN during the first, fifth, and sixth growth intervals (Fig. 4a). Middle size-range fish exposed to the LD 16:8 photoperiod had greater ($P<0.05$) growth rates during the third, fifth, and sixth growth intervals (Fig. 4b). However, LDN fish had significantly elevated growth rates over the course of the second growth interval. Two-way ANOVA revealed a significant ($P<0.001$) interaction between time and photoperiod on growth rate profiles for both the small and medium size-range fish. LD 16:8 middle size-range fish that eventually entered the UMG had significantly greater growth rates ($P<0.01$) throughout the experiment than those LD 16:8 fish that remained in the LMG. With the exception of the mid-September measurement interval, when the growth rate for LDN fish was significantly higher, the growth rates of the large size-range fish did not differ between photoperiods (Fig. 4c). There was no significant interaction between photoperiod and time on growth rates for the large size-class fish.

Mean plasma T_4 levels were significantly lower ($P<0.01$) in LD 16:8 fish from the UMG as compared with upper modal group LDN fish. However, T_3 levels and T_3/T_4 ratio were greater in fish from the LD 16:8 upper modal group (Table 1).

Discussion

Exposure to the LD 16:8 photoperiod resulted in a greater number of fish entering the UMG compared with fish maintained under LDN. Saunders et al. (1990) have also shown that exposure of juvenile Atlantic salmon to extended daylength starting in late August increased the proportion in the UMG. However, if the onset of extended daylength occurred in late September, the proportion of fish in the UMG did not differ from the natural photoperiod. Since UMG fish complete smoltification the following spring, exposure to an extended daylength in late August/early September presents an inexpensive means of increasing the proportion of smolts produced in 1, rather than 2 yr.

Villarreal et al. (1988) have shown that exposure of fish to natural photoperiods of 12-mo frequency which have been phase altered does not result in enhanced recruitment into the UMG. Fish held under 6- and 9-mo phase-delayed photoperiods had a smaller proportion in the UMG than fish held under LDN, whereas exposure to a 3-mo phase-delayed photoperiod had no effect on the percent of fish in the UMG when compared with LDN. Fish exposed to the 6- and 9-mo phase-delayed photoperiods experienced short, rapidly decreasing days in the summer which resulted in relatively poor feeding opportunities and, therefore, depressed growth rates. The depressed growth rates resulted in fewer fish reaching the threshold size to enter the UMG.

Since the CFL did not differ between photoperiods, it can be concluded that the LD 16:8 photoperiod had no effect on the process through which the bimodal length-frequency distribution occurred, but rather the LD 16:8 photoperiod increases the growth rate of the small and middle size-ranges of fish. This increase in growth rate allows a greater number of fish in the middle size-range to reach the CFL and enter the UMG.

The growth rate enhancing effect of LD 16:8 was size-dependent since the LD 16:8 photoperiod had no growth enhancing effect on fish in the large size-range. In contrast, exposure of a graded population of Atlantic salmon containing only UMG fish to an extended daylength of LD 16:8 in early October resulted in increased linear growth compared with fish under LDN (J. Duston and R. L. Saunders, unpubl. data). The increase in daylength in the above study involved an abrupt increase from natural daylength to the extended photoperiod with no previous period of declining photoperiod. These two contrasting observations are difficult to explain since previous reports of increased growth rates of juvenile salmon in response to extended daylength have examined entire populations of ungraded fish (Lundqvist 1980; Saunders et al. 1985). Perhaps the decrease in daylength at the start of our experiment inhibited a growth response in large size-classes.

Separation into modal groups is due to changes in feeding motivation and appetite (Higgins and Talbot 1985; Metcalfe et al. 1986, 1988). More specifically, UMG fish show an increase in appetite and adopt a fast development strategy, whereas fish in the LMG show a decrease in appetite and develop at a slower rate. The growth-stimulating effect of extended and/or increasing daylength on salmonids is thought to be due more to an increase in appetite than to changes in food conversion efficiency (Saunders and Henderson 1970; Clarke et al. 1978). Thus, it is likely that the LD 16:8 photoperiod increases the proportion of fish in the UMG by stimulating the appetite of fish in the middle size-class.

Based on studies on Scottish Atlantic salmon, Metcalfe et al. (1988) suggest that the differences in appetite, and therefore development, between fish in the UMG and LMG are fixed prior to September, with the timing of this divergence thought to occur soon after midsummer (Thorpe et al. 1980; Thorpe 1986). In contrast, our results show that it is possible to increase the number of fish entering the UMG with an environmental manipulation as late as September. The higher water temperatures of our laboratory, compared with conditions in Scotland, may be responsible for the extended time period over which fish can make the commitment to the UMG.

The depressed T_4 levels in the LD 16:8 fish agrees with Gross et al. (1965) who found decreased thyroid activity in green sunfish held under extended daylength. Brown and Stetson (1985) found an increased negative feedback sensitivity of the hypothalamo-pituitary axis in *Fundulus heteroclitus* exposed to long photoperiod. Further studies are needed to determine if feedback sensitivity is responsible for the lower T_4 values in Atlantic

salmon exposed to long days. Recent research has shown that T_3 is the more active of the thyroid hormones (Eales 1985). For example, plasma T_3 levels, and not T_4, are significantly correlated to specific growth rate in Arctic charr (Eales and Shostak 1985). One of the important regulatory pathways in the thyroid system is the conversion of T_4 to T_3 via the 5′-deiodinase enzyme. The higher T_3 levels and T_3/T_4 ratio in the LD 16:8 fish indicate an increase in conversion has occurred under the extended daylength.

In summary, the LD 16:8 photoperiod represents an inexpensive means of increasing the number of 1+ smolts. The extended daylength does not affect the process by which the bimodal distribution develops. Instead, it allows a greater number of fish in the middle size-range (6.8–7.7 cm) at the start of the experiment to reach the critical length and enter the UMG. Extended daylength also alters thyroid status.

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Phase Differences between Check Ring Locations in the Orange Roughy Otolith (*Hoplostethus atlanticus*)[1]

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Gauldie, R. W. 1990. Phase differences between check ring locations in the orange roughly otolith (*Hoplostethus atlanticus*). Can. J. Fish. Aquat. Sci. 47: 760–765.

Orange roughy (*Hoplostethus atlanticus*) otoliths were shown to have two different mineral phases of aragonite in the upper (anti-sulcul) and lower (sulcul) parts of medio-lateral sections of otoliths. The difference in mineral phase was characterised by differences in the direction of crystal growth, crystal size and crystal jointing. The effect of the difference in mineral phase was to change the patterns of homologous check rings observed in both the upper (anti-sulcul) and lower (sulcul) parts of the otolith. On average, about 24% of the information contained in check ring pattern of the upper (anti-sulcul) part of the otolith was retained in the lower (sulcul) part of the otolith. The loss of information may explain the difficulty in reconciling age interpretations made from sulcul check rings with age interpretations made from ultrastructural patterns in the anti-sulcul parts of medio-lateral sections of otoliths.

On a montré que les otolithes de l'hoplostète orange (*Hoplostethus atlanticus*) présentent deux phases minérales différentes de l'aragonite dans les parties supérieure (anti-sulcus) et inférieure (sulcus) des sections médiolatérales des otolithes. La différence dans les phases minérales est caractérisée par les différences dans la direction de croissance des cristaux, dans leur taille et dans leur jonction. Cette différence a pour effet de modifier la structure des cercles homologues observés à la fois dans la partie supérieure (anti-sulcus) et dans la partie inférieure (sulcus) de l'otolithe. En moyenne, environ 24 % de l'information fournie par la structure des cercles de la partie supérieure (anti-sulcus) de l'otolithe se trouve dans la partie inférieure (sulcus). La perte d'information peut expliquer la difficulté qu'on éprouve pour rapprocher l'interprétation de l'âge à partir des cercles de croissance du sulcus de celle que donne la configuration infrastructurale des parties anti-sulcus des sections médiolatérales des otolithes.

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Teleosts generally have three pairs of otoliths, large sagittae, and smaller lapilli and asterici, located in the inner ear and composed of calcium carbonate (usually in the aragonite morph) deposited on an organic matrix (Carlstrom 1963). Biological information is stored in various aspects of the ultrastructure and chemistry of otoliths. For example, the shapes of otolith, particularly the sagittae, have been used to identify species (Gaemers 1984; North et al. 1984); the chemistry of otoliths has been used to recover information about the fish environment (Gauldie et al. 1986; Radtke 1984); but most frequently, otoliths are used to determine the age of fishes.

Three kinds of periodic structures are present in otoliths, all of which have been used to estimate fish age: opaque and hyaline zones, daily growth increments, and check rings (Bagenal 1974). A convergent series of opaque and hyaline zones proceeding from the nucleus to the edge of otoliths is often observed at low magnification by either transmitted or reflected light (and often after heating for optical enhancement). The patterns formed by such series of zones are often interpreted to give the age of fish (Williams and Bedford 1974). Daily growth increments have been used to estimate the age of juveniles (Jones 1985) and some adult fish (Pulfrich and Griffiths 1988; Laurs et al. 1985; Ralston 1985). Unvalidated microscopic growth increments, assumed to be daily, have been used as a basis for age estimation in other species of fish (Ralston and Williams 1988; Campana and Neilson 1985; Ralston and Miyamoto 1983). Daily growth increments appear to form as a consequence of diurnal rhythms in the mobilisation of calcium (Mugiya 1987) and neuropeptide production (Gauldie and Nelson 1988). The check rings of otoliths have a more complicated origin.

The term check rings has been used to describe a variety of ultrastructural features of otoliths, as well as the optical effects that arise from them. The original usage of the term "check ring" arose when the banding patterns used to estimate age consisted of a series of fairly broad opaque and hyaline zones each pair of which defined an annulus in the radiating growth pattern of the otolith (Williams and Bedford 1974). Tong and Vooren (1972) used the term check ring to describe a narrow opaque zone "that did not fit in with the overall zonation pattern," following the usage of Hickling (1933). A more widespread usage of the term check ring (which is the usage followed in this paper) reflects the change in interpretation of what was an opaque zone in otoliths. This change occurred sometime between Dannevig's (1956) description of the effects of burning on what were clearly the opaque zones of cod otoliths, and the assumption that narrow opaque check rings in the burned otoliths of the sole and other fishes were equivalent annual marks

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to that of opaque zones (Christensen 1964) and that a wider hyaline zone and a very narrow check ring could also define an annulus. In subsequent papers, check rings, as opposed to opaque zones, were assumed to be annual and were described as occurring in both the medio-lateral and sulcul sections of otoliths (Wiedemann Smith 1968). An example of this change in interpretation can be seen by comparing the estimation of age in *Sebastes alutus* from opaque zones that form annuli in the otolith (Westrheim 1973) with age estimation from annuli defined by check rings in the sulcul part of the otolith (Beamish 1979).

Although the check rings of fish otoliths play a major role in the age determination of many species of fish, and their subsequent management, there is very little information published on either the composition or ultrastructure of otolith check rings. One view based on staining techniques (Albrechtsen 1968) was that check rings were simply very narrow, protein-rich opaque zones, and that perhaps through very slow growth of the otoliths, annual opaque zones became compressed to check rings. However, Gauldie (1988a) has shown that check rings are due to changes in crystalline structure, but there are no criteria to differentiate between annual check rings and accessory check rings (i.e. check rings that do not fit in with the overall zonation pattern (Keir 1960)).

While orange roughy otoliths show many opaque zones (Kotlyar 1980), the interpretation of such zones is complicated by surface sculpturing of the otolith (Gauldie 1988b). Sections of the orange roughly otolith typically contain many opaque zones whose patterns do not fit in with any overall zonation pattern (Gauldie et al. 1987). These occur in both the medio-lateral and sulcul aspect of the otolith.

The orange roughy is a taxonomically primitive fish, related to the berycids and diretmids (Rosen 1973). Unlike the otoliths of other trachichthids (and berycids and diretmids), the otolith of the orange roughy comprises but a single (occasionally a double) prism. Nearly all other teleost otoliths have a prismatic structure (Degens et al. 1969) similar to that of molluscs (Suzuki and Uozumi 1981), even though the prisms may be fully developed only in the adult (North et al. 1984).

The simplicity of the orange roughy otolith makes it a convenient model to trace the relationship between the ultrastructure of check rings in the sulcul side of the otolith, and those developed in the medio-lateral growth plane; and to reconcile the differences in the deposition patterns of such check rings.

Materials and Methods

A random sample of 10 orange roughy otoliths was taken from collections made in September 1986 on the Chatham Rise east of New Zealand and stored dry in envelopes at the Fisheries Research Centre, Wellington.

Sections of otoliths were made by cutting along the principal growth axis from the nucleus to the posterior edge with a diamond saw (Fig. 1a). The sections of otolith were mounted on glass slides or stainless steel pin-stubs with epoxy resin, and ground to the required thickness with a Struers Pedemax Petrographic grinder using 4 Im grit wet-and-dry paper.

Otoliths were prepared for scanning electron microscopy (SEM) by breaking with thumb pressure, mounting on stainless steel pin-stubs with epoxy resin and sputter-coated with gold.

Where necessary, etching of otoliths was carried out in a 0.1 m EDTA (ethylene diamino tetra acetic acid) pH 2.5 for 2 min.

Otoliths were examined by X-ray powder diffraction. Samples were ground in an agate mortar and pestle and analysed on a Phillips PW1279/PW1710 instrument under the following operating conditions: Cu tube at 45 kV, 40 mA automatic divergence slit, 0.1 mm receiving slit, $1\frac{1}{2}$ scatter slit and Ni filter. Associated errors were $\pm 5\%$.

The terminology for the crystalline forms of aragonite was that of Carriker et al. (1980).

Results

All of the otoliths used in this study were shown by X-ray diffraction to consist entirely of aragonite.

Broken orange roughy otoliths showed a boundary (Fig. 1b) between the lower sulcul section and the upper, anti-sulcul part of the otolith, the region described as containing the principal

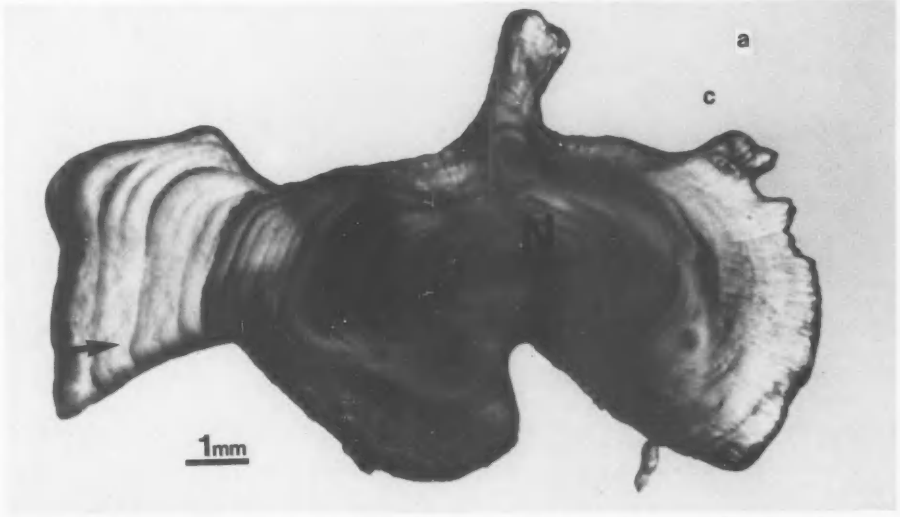

FIG. 1. Orange roughy otolith: (a) A whole otolith shows the line of section (arrows) from the nucleus (N) to the posterior edge.

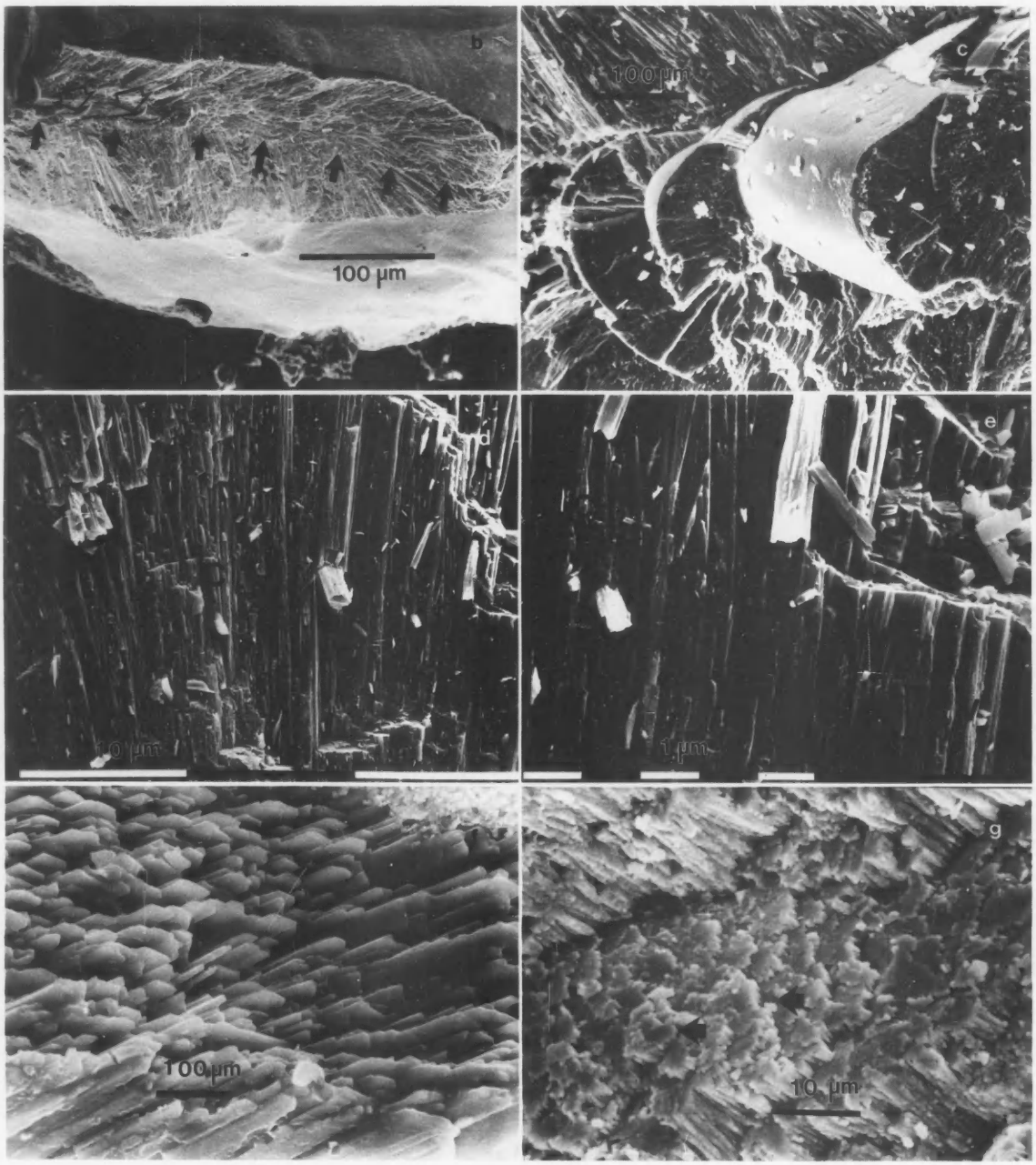

FIG. 1. Orange roughy otolith: (b) a boundary (arrows) lies between the upper (anti-sulcus) section and the lower (sulcul) section. Spalling lines (open arrows) are characteristic of the anti-sulcul section; (c) spalling lines (arrow) have a characteristically smooth surface; (d) check rings sometimes appear as fine cracks (C) or discontinuities in the crystal structure; (e) at higher magnification the check rings (C) fade into the lattice anomaly (A) and eventually disappear (V); (f) the crystals at the growing (anti-sulcul) top of the otolith have a lath-like structure; (g) broken crystals with the characteristic terminating screw dislocation of aragonite.

growth axis (Gauldie et al. 1987) or maximum growth axis (Pannella 1980) and referred to here as the medio-lateral section. The boundary marked a difference in the direction of crystal growth in the anti-sulcul and sulcul sections of the otolith (Fig. 1b).

The anti-sulcul section of the otolith showed spalling lines (Fig. 1b). Spalling lines have been demonstrated in otoliths from a number of different species (R. W. Gauldie, unpubl. data) and all showed a characteristically fine grained crystalline surface contrasting with that of the monoclinal aragonite crystals as was found in the anti-sulcul section of the orange roughy otolith (Fig. 1c). Spalling lines ranged from the gross structures of Fig. 1c to fine cracks in the otolith (Fig. 1d) which gradually faded out into a kind of lattice anomaly and finally disappeared (Fig. 1e). The monoclinal crystals that characterised the anti-sulcul section of the otolith showed a lath-like appearance when they were observed at the growing tip of the otolith (Fig. 1f). Broken parts of the anti-sulcul section that did not fall at spalling lines, showed the terminating screw dislocations (Fig. 1g) characteristic of aragonite (Degens 1976).

The sulcul section of the otolith showed crystals growing almost orthogonal to the direction of growth of crystals in the medio-lateral section (Fig. 1b). In addition, the crystals in the sulcul section showed a jointed habit (Fig. 2a). The sulcul crystals observed at the growing edge close to the sulcus had a different habit from those in the medio-lateral section, ranging from thickened laths (Fig. 2b) to hexagon-like prisms (Fig. 2c). The size of crystals in the sulcus aspect ranged from slightly larger (1.2×) to about twice the thickness of crystals in the medio-lateral aspect. Spalling lines were never observed in the sulcul aspect of the orange roughy otolith, but occasionally crack-like structures appeared in some broken sections (Fig. 2d) that may be artefacts. The broken crystals of the sulcus section of the otolith did not show the terminating screw dislocations characteristic of aragonite (Fig. 2d).

Sections which had been polished and etched showed check rings in the anti-sulcul section passing through the boundary layer into the sulcul section of the otolith (Fig. 2e). Check rings could be traced from the anti-sulcul section into the sulcul section of the otolith. In some cases, particularly in the thicker sulcul sections of larger otoliths, sporadic sequences of micro increments of the same order of magnitude as daily growth increments appear (Fig. 2f) with indications of fusion of both check rings and micro increments.

The pattern of zonation is defined by the distance between check rings (or, at low magnification, clusters of check rings). Therefore, the patterns of zonation caused by the same check rings appearing in both the anti-sulcul and sulcul sections of the otolith could be compared. The simplest basis of comparison was the coefficient of correlation of the distances between homologous check rings in both sections of the otolith.

Sections of 10 otoliths were examined and a sequence of about 20 consecutive check rings measured in both the medio-lateral and sulcul sections of each otolith. Correlation coefficients ranged from 0.167 to 0.789, with a mean of 0.49, which indicated that (on average) only about 24% of the information (i.e. distance between check rings) in the zonation pattern was retained by homologous check rings as they passed from the medio-lateral to the sulcul aspect of the otolith. In effect, two different patterns of zonation were produced by homologous check rings in the anti-sulcul and sulcul aspects of the otolith, respectively.

Discussion

Aragonite in teleost otoliths occurs in the twinned form (Gauldie and Nelson 1988). Twinning is characteristic of other biogenic aragonites (Marsh and Sass 1980). Twinning results in a single, large, three-dimensional crystal grown from a single focus. The orange roughy (Hoplostethus atlanticus) otolith is unusual in usually consisting of a single prism and is consequently a single crystal of aragonite. Within this crystal, the longest of the twinned crystals, the radial twin, is that growing directly from the nucleus to the posterior edge of the otolith, the principal growth axis. This crystal twin can be followed along the principal growth axis in Fig. 1b. En route, the radial crystal develops medial twins that grow from the centre of the growth axis towards the sulcul and anti-sulcul surfaces of the otolith. Consequently, the crystal at the growing tip of the growth axis is the twin of the same crystals that grow directly from the nucleus to the sulcul and anti-sulcul surfaces of the otolith. Therefore, by definition, the crystals at the surface of the sulcus (and the anti-sulcus surface) must be of exactly the same age as the crystal at the growing tip of the otolith.

The orange roughy otolith is divided into an upper (anit-sulcul) part and a lower (sulcul) part by a distinct optical boundary that is accompanied by a change in the mineralogy of the crystals. The crystals of the sulcul part of the otolith have a different mineralogy to those in the anti-sulcul part of the otolith. The changes in the habit of aragonite crystals in the sulcul section, particularly size, indicate that these crystals are growing more slowly than the crystals in the medio-lateral section (De Jong 1959) which is what one would expect from a shorter twin crystal of the same age.

These observations show that a change occurs between the mineralogies of the medio-lateral and sulcul sections in the otolith of the orange roughy. The cause of the phase change appears to be related to the growth rate of crystals in both sections of the otolith, but there may be other factors (e.g. matrix changes related to macular neuro-secretion) beyond the scope of ultrastructural studies. Whatever the cause of the phase change, the effect is to drastically alter the separate patterns of zonation formed by homologous check rings in the anti-sulcul and sulcul sections of the otolith, respectively. These alterations in the pattern of zonation lead to less than a quarter of the "pattern" information in the anti-sulcul section persisting in the sulcul section.

The pattern of opaque and hyaline zones in the anti-sulcul section of the otolith of the oyster toadfish *Opsanus tau* appears to reflect the seasonal change in width of daily micro-increments (Radtke et al. 1985). There are strong physiological arguments (Gauldie and Nelson 1988; Mugiya 1987) that daily deposition of micro-increments are tied to the diel rhythms of the brain of fishes, and are therefore an obligatory feature of otolith growth in most fishes for most of their life cycle. These arguments provide a physiological mechanism for the argument that opaque and hyaline zones are markers of seasonal changes in the growth rate of otoliths. Thus the information in the anti-sulcul section that is used to estimate age of fish is on a firm scientific footing which would allow one to confidently assay the validity of the patterns of opaque and hyaline zonation in the anti-sulcul section of the otolith.

The discontinuities that appear in the principal growth axis of the orange roughy otolith are in the form of crack-like structures and spalling lines. The crack-like structures observed in the orange roughy otolith were similar to those reported in other

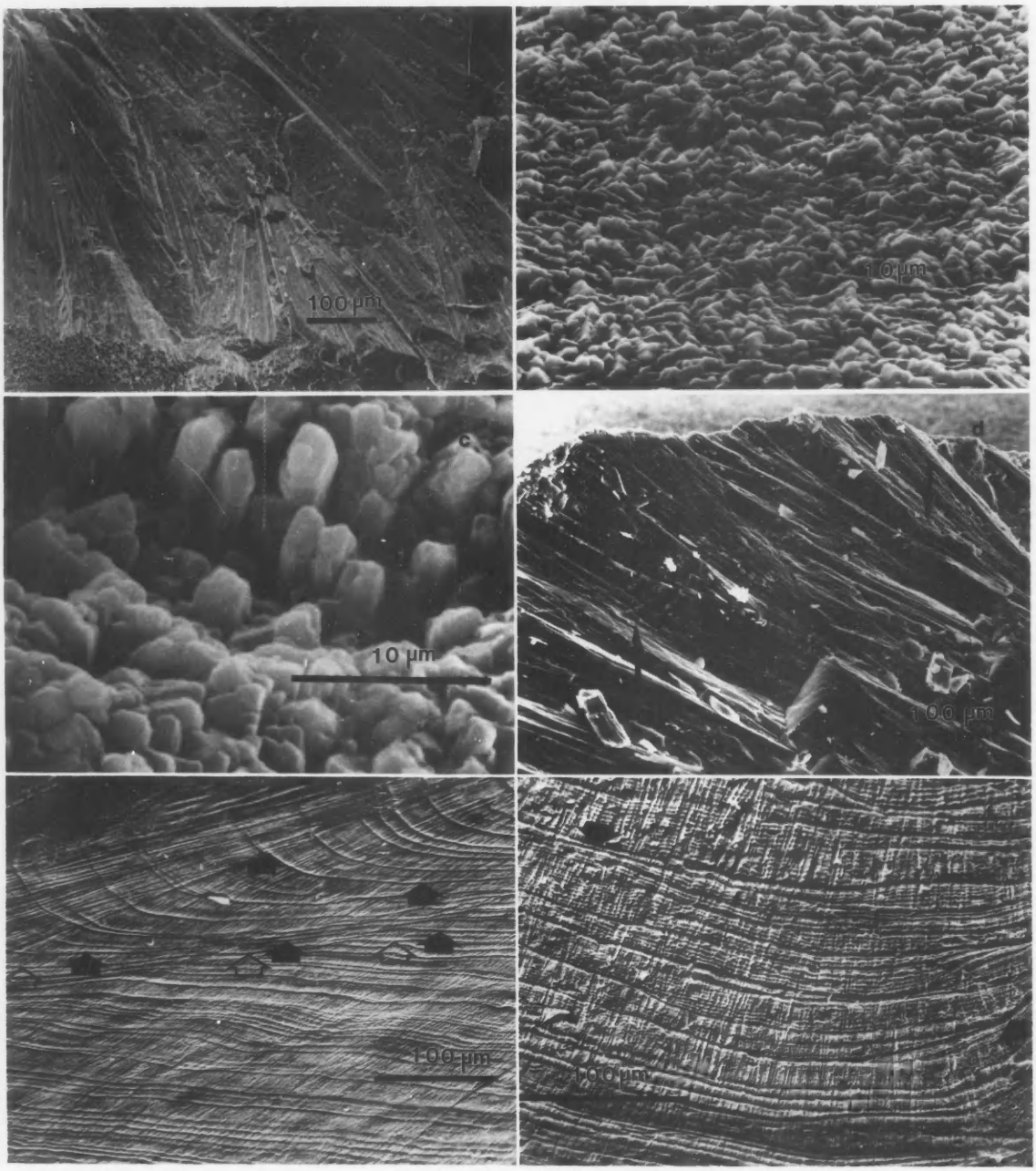

FIG. 2. Sulcul sections of orange roughy otolith: (a) crystals with a jointed habit; (b) crystals at the sulcul growing face show differences in habit ranging from thickened laths, to (c), hexagon-like prisms; (d) spalling lines did not occur in the sulcul section but crack-like structures (arrows) were observed in some sections. Broken sections of crystals were smooth (open arrows) without signs of terminating screw dislocations; (e) polished and etched sections showed check rings which could be traced (arrows) from the anti-sulcul to the sulcul sections (f) in some polished and etched sections micro-increments could be seen between check rings in the sulcul aspects of the otolith, as well as fusion of check rings (arrows).

otoliths (Degens et al. 1969). The crystals on either side of the cracks have a remarkable fidelity in both width and direction that makes it unlikely that the otolith has stopped growing, and then restarted, because restarting crystal growth requires renucleation and a progression of small to larger sized crystals. Spalling lines, or lenses of finer crystals, are more difficult to explain, but can only arise from a change in growth rate of the crystal. Spalling lines occur only in the principal growth axis so that if they do represent any pause in growth, it can only make the radial twin at the tip of the otolith older, not younger, than the medial twin at the sulcul surface.

The sulcul section of the orange roughy otolith does not have a continuous deposit of micro-increments that might generate seasonal patterns of zonation equivalent to the anti-sulcul patterns and thus provide a physiological mechanism for the pattern of sulcul check rings. Nonetheless, check rings observed in the sulcul section of the orange roughy otolith are homologous to those observed in the anti-sulcul section. However, the patterns of homologous check rings in the sulcul section becomes so distorted as to be unrecognizable from that in the medio-lateral section. One aspect of the distortion is to make the spacing between check rings in the sulcul part of the otolith appear more regular than that between homologous check rings in the anti-sulcul section.

Thus even in an otolith of simple design, such as the orange roughy sagitta, there is a loss of information in check ring patterns due to a change in both the spacing and phase of the patterns of zonation in the anti-sulcul and sulcul sections caused by differences in mineralogy between faster and slower growing twins of the same aragonite twinned crystal. This loss of information makes the interpretation of the patterns of zonation in both sections of the otolith difficult to reconcile.

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Tissue Distribution and Renal Excretion of Ormetoprim after Intravascular and Oral Administration in the Channel Catfish (*Ictalurus punctatus*)

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Ormetoprim is used to potentiate sulfadimethoxine in treating certain bacterial diseases of aquatic species. The tissue disposition and renal excretion of ormetoprim and metabolites were examined after intravascular and oral administration (4 $mg \cdot kg^{-1}$) in channel catfish (*Ictalurus punctatus*). Peak plasma level (0.66 $\mu g \cdot mL^{-1}$) of ^{14}C-ormetoprim occurred at 6 h after oral dosing. The oral bioavailability was estimated at 52%. Ormetoprim and metabolites were widely distributed in the tissues. The tissue concentrations were highest in the liver, trunk kidney, head kidney, and spleen. Clearance of the radiolabel from tissues was rapid. The muscle contained 49.3% of the intravascularly administered dose at 2 h; however, at 72 h, less than 1% of the dose remained in this tissue. ^{14}C-Ormetoprim was more persistent in the skin than in the muscle. Ormetoprim was extensively metabolized in catfish. After intravascular administration, 21.1% of the dose of ^{14}C-ormetoprim was eliminated in the urine in 48 h, predominantly as polar metabolites; less than 4% of the dose was eliminated as the parent compound. Biliary excretion was a minor route of elimination (5–6% of the dose). The data suggest branchial excretion of ormetoprim and/or metabolites.

L'ormétoprim est un activateur de la sulfadiméthoxine dans le traitement de certaines infections bactériennes d'espèces aquatiques. Le dépôt dans les tissus et l'excrétion rénale de l'ormétoprim et de ses métabolites ont été suivis après l'administration par voie orale ou intravasculaire (4 $mg \cdot kg^{-1}$) à des barbues de rivière (*Ictalurus punctatus*). La concentration maximale dans le plasma (0,66 $\mu g \cdot mL^{-1}$) de ^{14}C-ormétoprim a été observée 6h après administration par voie orale. La biodisponibilité du produit administré sous forme orale a été évaluée à 52 %. L'ormétoprim et ses métabolites sont largement distribués dans les tissus. Les concentrations tissulaires le plus élevées ont été observées dans le foie, l'opistonephros, le pronéphros et la rate. La clearance tissulaire du produit radiomarqué est rapide. Deux heures après l'administration intravasculaire, le tissu musculaire contenait 49,3 % de la dose; après 72 h cependant, ce tissu n'en contenait plus que moins d'un p. cent. Le ^{14}C-ormétoprim s'est révélé plus persistant dans la peau que dans le tissu musculaire. L'ormétoprim est largement métabolisé chez la barbue. Après l'administration intravasculaire, 21,1 % du ^{14}C-ormétoprim est éliminé dans l'urine au bout de 48 h, principalement sous forme de métabolites polaires. Moins de 4 % de la dose est éliminé sous la forme du composé-parent. L'excrétion biliaire constitue une voie mineure d'élimination (5 à 6 % de la dose administrée). Les résultats indiquent qu'il se produit une excrétion branchiale de l'ormétoprim ou de ses métabolites.

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The diaminopyrimidine drug ormetoprim is used in combination with sulfadimethoxine for its potentiating effect in the treatment of certain bacterial diseases in fish. The sulfadimethoxine:ormetoprim (5:1) combination is presently approved by the United States Food and Drug Administration (FDA) for treatment of furunculosis (caused by *Aeromonas salmonicida*) in salmonids and enteric septicemia (caused by *Edwardsiella ictaluri*) in channel catfish (*Ictalurus punctatus*). This combination of drugs was found effective in field trials when administered at 50 $mg \cdot kg^{-1} \cdot d^{-1}$ for five consecutive days (Bullock et al. 1974; Bullock et al. 1983a; Plumb et al. 1987). Efficacy has also been demonstrated in the treatment of infections caused by *Aeromonas hydrophila* in channel catfish (Bowser et al. 1987) and *Yersinia ruckeri* in salmonids (Bullock et al. 1983b).

Tissue residue and therapeutic efficacy studies are required by the FDA for drug approval in aquatic food species. The tissue disposition of sulfadimethoxine has been well characterized

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in channel catfish (Squibb et al. 1988), rainbow trout (*Oncorhynchus mykiss*) (Kleinow and Lech 1988) and American lobster (*Homarus americanus*) (Barron et al. 1988). The pharmacokinetics of ormetoprim in rainbow trout have also been reported (Droy et al. 1989). The present study examines the bioavailability, tissue distribution, and renal excretion of ormetoprim and metabolites in channel catfish.

Materials and Methods

Experimental Animals and Dosing

Pond-reared channel catfish, 0.5–1 kg body weight, were individually distributed and acclimated in aerated, 90-L aquaria. Water temperature was 28 ± 1°C.

^{14}C-Ormetoprim (specific activity, 52.2 kBq·mg^{-1}) was obtained from Hoffman-La Roche Inc. (Nutley, NJ). The compound was dissolved in a mixture of ethanol and Emulphor (GAF Corp., New York, NY) and then diluted with 0.85% sodium chloride to yield a solution of 8 mg·mL^{-1} (417.6 kBq·mL^{-1}) in ethanol:Emulphor:0.85% sodium chloride (3:4:3). This solution was administered at a dosage of 0.5 mL·kg^{-1} (4 mg·kg^{-1}; 208.8 kBq·kg^{-1}) to anesthetized fish either intravascularly via the caudal vein or orally in gelatin capsules (Plakas et al. 1988).

Tissue and Fluid Distribution Study

Before drug administration, fish were catheterized for collection of urine using the balloon-catheter system described by Plakas et al. (1988). The efficiency of this method for urine collection has been proven with model compounds for renal clearance (S. M. Plakas, unpubl. data).

After intravascular or oral dosing, blood specimens were drawn from the caudal peduncle of anesthetized animals at frequent intervals and the plasma was separated for analysis. Urine and aliquots of tank water were collected at 2, 4, 24, and 48 h after dosing. At 2, 4, 24, 48, and 72 h after dosing, five to seven fish were killed and the following tissues collected: muscle, liver, head kidney, trunk kidney, spleen, and skin. Bile was taken by syringe from the gall bladder. The entire gastrointestinal tract was excised and the contents were flushed with 50 mL of water.

All tissues, except muscle and skin, were removed and weighed. Muscle and skin were assumed to comprise 50 and 6% of the body weight, respectively. Plasma volume was estimated from the actual hematocrit values and the assumption that whole blood comprises 5% of body weight. Tissue specimens, biological fluids, and tank water were analyzed in duplicate for total radioactivity. Concentration data were expressed in ^{14}C-ormetoprim equivalents per unit weight or volume. The percentage of the dose remaining in each tissue or fluid over time was calculated from the total tissue weight or fluid volume and the concentration of the radiolabel.

Renal Excretion Study

An additional study was performed to characterize the renal excretion of parent ormetoprim and metabolites using catheters that were extended outside of the tank. Urine was continuously collected from four fish after intravascular dosing and five fish after oral dosing. Collection intervals were 2, 4, 8, 12, 16, 20, 24, 30, 36, 42, and 48 h. Urine specimens were analyzed for parent compound and total radiolabel. Aliquots of tank water were analyzed for total radioactivity.

Uptake from Water

To examine the potential for absorption of excreted ormetoprim and/or metabolites, ^{14}C-ormetoprim was added at the usual dosage (4 mg·kg^{-1} body weight) directly to the water of six aquaria, each of which contained a single fish. Several additional fish were placed in tanks previously occupied (for 72 h) by fish that had been given an oral dose of ^{14}C-ormetoprim. Tissues, urine, bile, and tank water were collected and analyzed for the radiolabel at 2, 4, 24, 48, and 72 h.

Analytical Procedures

Scintillation counting was performed using a Packard Tri-Carb Liquid Scintillation Spectrometer Model 2425 (Packard Instrument Co., Downers Grove, IL). Tissues, bile, and gut contents were analyzed in duplicate using NCS tissue solubilizer and OCS counting scintillant (Amersham Corp., Arlington Heights, IL). The scintillation cocktail was neutralized with glacial acetic acid before counting. Aliquots of urine and tank water were analyzed using Ecolume (ICN Biomedicals, Inc., Irvine, CA) or Ready Safe (Beckman Instruments, Inc., Fullerton, CA) cocktail. Quench curves were generated by the external standard ratio method.

Urine specimens collected in the renal excretion study were analyzed for parent compound and for distribution of total radioactivity as polar and nonpolar compounds. Ormetoprim analysis followed the HPLC procedure of Weiss et al. (1987). A 0.5-mL aliquot of urine was mixed with pH 10 buffer (Fisher Scientific, Fair Lawn, NJ) and extracted with 1 mL of methylene chloride by vortex mixing for 2 min. Aliquots (0.2 mL) of the separated organic and aqueous phases were transferred to scintillation vials for radioassay of total nonpolar and polar compounds. The organic solvent was evaporated before adding scintillant. The remainder of the organic phase in the extraction procedure was transferred to a sealed vial and stored under refrigeration until HPLC analysis (within 24 h). The extraction efficiency of ^{14}C-ormetoprim from spiked control urine was 98%.

The HPLC system consisted of an SP8700 LC pump, SP8480 UV detector (288 nm), and SP4270 integrator (Spectra Physics, San Jose, CA). A Waters μ Porasil 3.9 mm × 30 cm stainless steel column (Millipore Corp., Milford, MA) equipped with a 4.6 × 10 mm guard column was allowed to equilibrate overnight with mobile phase of chloroform:methanol:water: ammonium hydroxide (500:14:1:0.3), flow rate 1.5 mL·min^{-1}. A 250 μL aliquot of the urine extract was manually injected. Ormetoprim eluted in approximately 5 min (total run time 10 min). There were no interfering peaks in control urine. The overall recovery of ormetoprim from spiked urine (compared with external standards) on HPLC was 105%. The limit of determination of parent ormetoprim in the urine was 0.1 μg·mL^{-1}.

To confirm and further characterize the renal excretion of ormetoprim and metabolites, representative urine specimens were analyzed by an additional HPLC procedure. Urine specimens were evaporated to dryness and the residue was dissolved in a mobile phase consisting of acetonitrile:0.022 M potassium phosphate monobasic solution (2:8) at pH 7.9. A Waters M-45 pump and RCM-100 Radial Compression Module (C_{18} column insert) was used with a column flow rate of 1.5 mL·min^{-1}. Fractions were collected at 30-s intervals and analyzed for radioactivity. Authentic metabolites of ormetoprim were not available.

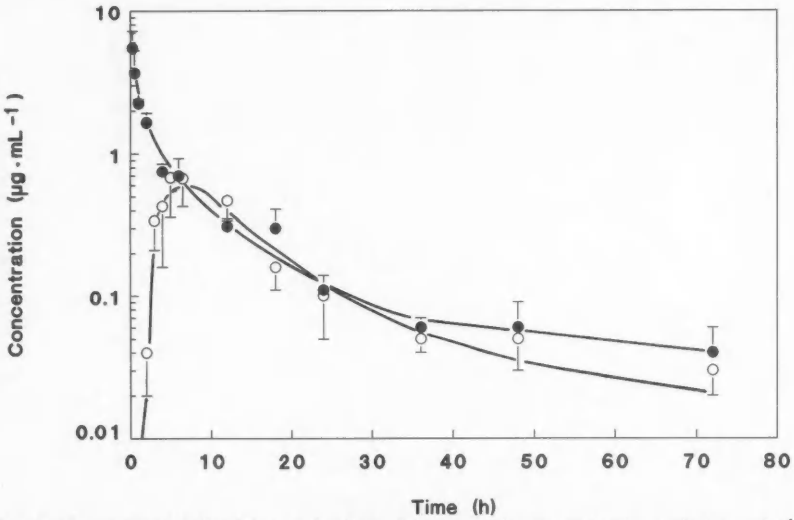

FIG. 1. Plasma concentration (± SD) of radiolabel (expressed in μg ormetoprim equivalents·mL^{-1}) after intravascular (-●-) and oral (-○-) administration (4 mg·kg^{-1}) of ^{14}C-ormetoprim in channel catfish.

TABLE 1. Mean tissue concentrations ± SD (as μg equivalents of ormetoprim per g or mL) and recovery of the dose (percent, in parentheses) of ^{14}C-ormetoprim after intravascular administration (4 mg·kg^{-1}) in channel catfish.

	Time after dosing (h)				
Tissue	2	4	24	48	72
Liver	17.50 ± 2.23 (4.5)	9.98 ± 2.66 (2.7)	1.24 ± 0.41 (0.4)	0.65 ± 0.35 (0.2)	0.43 ± 0.23 (0.1)
Head kidney	12.83 ± 2.54 (0.6)	7.22 ± 2.20 (0.4)	2.08 ± 0.92 (0.1)	1.67 ± 0.93 (0.1)	0.88 ± 0.43 (<0.1)
Trunk kidney	12.36 ± 1.59 (1.6)	6.61 ± 1.83 (0.9)	1.99 ± 0.66 (0.3)	1.14 ± 0.41 (0.2)	0.91 ± 0.33 (0.1)
Spleen	8.55 ± 1.88 (0.2)	4.37 ± 1.76 (0.1)	1.72 ± 0.71 (<0.1)	0.98 ± 0.20 (<0.1)	0.51 ± 0.19) (<0.1)
Skin	4.45 ± 0.49 (7.1)	3.54 ± 0.43 (5.7)	1.42 ± 0.46 (2.3)	1.02 ± 0.41 (1.7)	0.74 ± 0.32 (1.2)
Muscle	3.95 ± 0.54 (49.3)	1.93 ± 0.33 (24.3)	0.20 ± 0.05 (2.5)	0.08 ± 0.04 (1.0)	0.04 ± 0.02 (0.6)
Plasma	1.66 ± 0.27 (1.4)	0.75 ± 0.10 (0.7)	0.11 ± 0.03 (0.1)	0.06 ± 0.03 (<0.1)	0.04 ± 0.02 (<0.1)

Pharmacokinetic Analysis

At most sampling times, the concentration of parent compound in the plasma was below the limit of detection by the HPLC analytical procedure. Thus, plasma data were modeled using total radiolabel data (ormetoprim and metabolites). Weighted plasma values were fitted using PCNONLIN software (Statistical Consultants, Inc., Lexington, KY). An apparent bioavailability of radiolabel was calculated on the basis of area under the plasma concentration-time curves for both routes of administration. The limit of determination of parent ormetoprim by HPLC was lower in the urine than in the plasma (i.e. differences in specimen volumes available). This enabled an estimate of the bioavailability of the parent compound on the basis of its renal excretion. Bioavailability was calculated from the cumulative percentage of the dose of ormetoprim excreted intact in the urine after oral and intravascular administration.

Results and Discussion

Plasma concentration-time ($C_p - t$) data for radiolabel after intravascular dosing (Fig. 1) were well described by the triexponential equation:

$$C_p = 5.95e^{-1.77t} + 1.39e^{-0.143t} + 0.108e^{-0.014t}$$

The model indicated that ^{14}C-ormetoprim was distributed between a central compartment, from which the drug is eliminated, and both shallow and deep peripheral compartments. The half lives for α, β and γ phases were 0.39, 4.9, and 49 h, respectively. In rainbow trout, a biexponential model described the plasma disposition of ^{14}C-ormetoprim, with $t_{1/2}\alpha$ and $t_{1/2}\beta$ of 0.54 and 17.54 h, respectively (Droy et al. 1989).

Total clearance from the body (Cl_b) was 192 mL·h^{-1}·kg^{-1}. The intercompartmental transfer clearances (Cl_t) of ormetoprim and metabolites for shallow and deep peripheral compartments

TABLE 2. Mean tissue concentrations ± SD. (as μg equivalents of ormetoprim per g or mL) and recovery of the dose (percent, in parentheses) of ^{14}C-ormetoprim after oral administration (4 mg·kg^{-1}) in channel catfish.

Tissue	Time after dosing (h)				
	2	4	24	48	72
Liver	1.80 ± 0.72 (0.7)	6.40 ± 3.73 (2.8)	1.92 ± 1.01 (0.7)	0.56 ± 0.24 (0.2)	0.30 ± 0.06 (0.1)
Head kidney	0.93 ± 0.53 (0.1)	5.50 ± 2.39 (0.2)	3.05 ± 1.32 (0.1)	1.43 ± 0.71 (0.1)	0.73 ± 0.34 (<0.1)
Trunk kidney	1.14 ± 0.31 (0.2)	6.45 ± 2.16 (0.9)	3.94 ± 1.77 (0.5)	1.35 ± 0.64 (0.2)	0.63 ± 0.14 (0.1)
Spleen	0.73 ± 0.38 (<0.1)	3.74 ± 1.49 (0.1)	2.66 ± 0.93 (0.1)	1.26 ± 0.55 (<0.1)	0.60 ± 0.19) (<0.1)
Skin	0.12 ± 0.10 (0.2)	1.02 ± 0.44 (1.6)	1.47 ± 0.67 (2.4)	1.10 ± 0.47 (1.8)	0.71 ± 0.21 (1.1)
Muscle	0.03 ± 0.01 (0.4)	0.80 ± 0.46 (9.8)	0.23 ± 0.11 (2.8)	0.06 ± 0.03 (0.7)	0.04 ± 0.01 (0.5)
Plasma	0.04 ± 0.02 (0.1)	0.43 ± 0.27 (0.3)	0.11 ± 0.05 (0.1)	0.03 ± 0.02 (0.1)	0.02 ± 0.01 (0.1)

TABLE 3. Percent of the intravascularly (and orally) administered dose of ^{14}C-ormetoprim recovered as total radioactivity in the tissues, fluids, gut contents, and tank water of channel catfish in the biodistribution study.

Compartment	Time after dosing (h)				
	2	4	24	48	72
Tissues[a]	64.7 (1.7)	34.8 (15.7)	5.7 (6.7)	3.2 (3.1)	2.0 (1.9)
Bile	1.6 (0.1)	2.6 (1.3)	5.7 (5.8)	5.9 (5.6)	5.5 (4.6)
Urine[b]	1.4 (0.1)	5.2 (1.1)	17.6 (19.3)	25.3 (29.5)	NS[c] (NS)
Gut contents	1.1 (77.6)	1.1 (40.5)	0.8 (1.4)	0.3 (0.4)	0.2 (0.2)
Tank water	12.7 (2.2)	27.4 (9.1)	60.4 (54.2)	74.5 (63.8)	NS (NS)

[a]Combined percent of the dose recovered from liver, head kidney, trunk kidney, spleen, skin, muscle, and plasma.
[b]Cumulative percent of the dose excreted as total radioactivity.
[c]NS, not sampled.

were 494 and 86 mL·h^{-1}·kg^{-1}, respectively. The apparent volume of distribution of the central compartment was 537 mL·kg^{-1} and indicated initial distribution of radiolabel outside the vascular system. The apparent volumes of distribution of the shallow (1061 mL·kg^{-1}) and deep (3905 mL·kg^{-1}) peripheral compartments were very large, suggesting wide distribution and concentration in the tissues. In rainbow trout, the apparent volume of distribution (V_{ss}) of ^{14}C-ormetoprim was 4854 mL·kg^{-1} (Droy et al. 1989). The $V_{d(area)}$ for parent ormetoprim was 1450 mL·kg^{-1} in cattle (Wilson et al. 1987). In the horse, the V_{ss} for ormetoprim was 1190 mL·kg^{-1} (Brown et al. 1989).

The peak plasma level (0.66 μg·mL^{-1}) of radiolabel occurred at 6 h after oral administration (Fig. 1). The absorption rate constant (k_a) was 0.35 h^{-1} and absorption half-life was 2 h. The apparent bioavailability of total radiolabel was 52%. In rainbow trout, the t_{max} of ^{14}C-ormetoprim was 12 h and the apparent bioavailability was 87% (Droy et al. 1989).

The patterns of tissue distribution of ormetoprim and metabolites were similar following intravascular and oral dosing (Tables 1 and 2). Ormetoprim and metabolites were concentrated in all tissues relative to plasma, particularly in the excretory tissues (i.e. liver and trunk kidney), head kidney and spleen. The unusual distribution in head kidney and spleen may have been the result of reversible binding to specific proteins. These tissues are sites of hematopoiesis where high dihydrofolate reductase activity would be expected. The mechanism of action and usefulness of ormetoprim relates to its ability to inhibit this enzyme in bacteria and other microorganisms but not in their mammalian and, presumably, piscine hosts.

Ormetoprim and metabolites were rapidly eliminated from most of the tissues analyzed. After intravascular dosing, the percentage of the dose recovered in the tissues decreased from 64.7% at 2 h to 2.0% at 72 h (Table 3). The muscle contained the largest proportion (49.3%) of the intravascularly administered dose at 2 h (Table 1). Clearance of radiolabel from the muscle also was rapid; at 72 h, 0.6% of the dose remained. Ormetoprim and metabolites persisted in the skin relative to the muscle and plasma. At 72 h, the skin contained 1.2% of the administered dose. The skin may represent the deep peripheral compartment from which ormetoprim and metabolites are more slowly released.

Previous studies in fish have demonstrated accumulation of ormetoprim and related compounds in the skin. The persistence of ormetoprim in the skin of salmonids was first reported by Bullock et al. (1983a). Subsequently, Droy et al. (1989) reported concentrations (total radioactivity) of 0.9 and 0.15 μg·g^{-1} in the skin and muscle, respectively, at 38 d after oral administration of ormetoprim at 8 mg·kg^{-1} in rainbow trout. In the present study, the levels in the skin and muscle of catfish were 0.74 and 0.04 μg·g^{-1}, respectively, at 3 d after oral dosing (4 mg·kg^{-1}). Accumulation in the skin also was demonstrated with the related compound trimethoprim in rainbow trout. An affinity of this compound for melanin was suggested (Bergsjo et al. 1979). Ormetoprim did not persist in the skin of chickens and turkeys (Fellig et al. 1972).

The skin of salmonid fishes, unlike that of catfish, typically is consumed by humans. The drug withdrawal times recommended by the FDA reflect the differences in residual persistence in the edible portions between these species. After treatment with the combination drug sulfadimethoxine:ormetoprim (50 mg·kg^{-1}·d^{-1} for 5 d), the withdrawal time

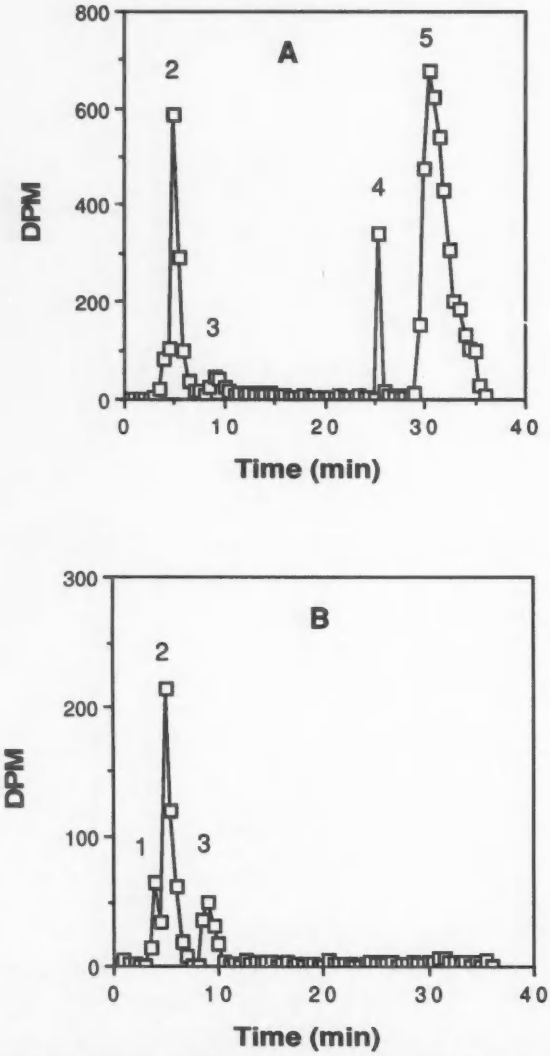

FIG. 2. HPLC profile of representative urine specimens from intravascularly dosed fish in the renal excretion study. Urine was collected during 0–2 h (A) and 20–24 h (B) intervals. Peaks 1–3 represent polar metabolites, peak 4 was a nonpolar metabolite and peak 5 was identified as parent ormetoprim. Each point represents cumulative radioactivity eluting in a 30-s period.

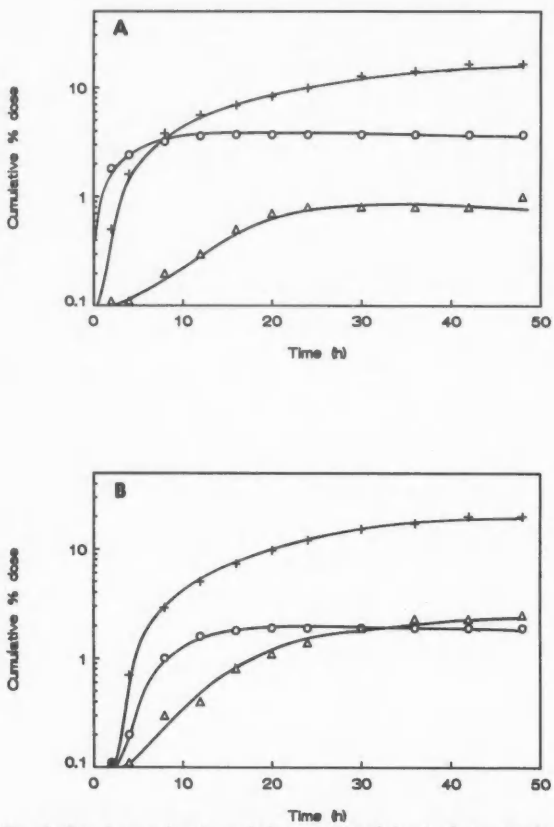

FIG. 3. Renal excretion (cumulative percent of dose) of ormetropin (-○-), nonpolar metabolites (-Δ-), and polar metabolites (-+-) following ^{14}C-ormetoprim administration in channel catfish. (A) Intravascular administration, (B) oral administration.

before harvest for human consumption is 42 d for salmonids and 3 d for channel catfish. The tolerance limit for ormetoprim in the edible portion is 0.1 $\mu g \cdot g^{-1}$. Based on available data for these species, adherence to recommended treatment and withdrawal times is critical in ensuring acceptable residue levels.

Ormetoprim was extensively metabolized by catfish (Figs. 2 and 3). HPLC analysis of the urine indicated the production of a single nonpolar and several polar metabolites. The cumulative percentages of the dose excreted as ormetoprim, nonpolar and total polar metabolites in 48 h were 3.7, 1.0, and 16.4%, respectively, after intravascular administration and 1.9, 2.5, and 20.0%, respectively, after oral administration. Measurable renal excretion of parent compound did not occur beyond 16 h, whereas elimination of the radiolabel as polar metabolites continued for up to 42 h. The renal excretion data suggested first-pass metabolism after oral administration. The bioavailability of ormetoprim in channel catfish, based on urinary elimination of the parent compound, was 51%.

The nonpolar metabolite isolated by HPLC may be *N*-acetylated ormetoprim; this supposition is based on its polarity and the preponderance of *N*-acetylation in the metabolism of primary amines in vertebrates (DeVree and Hekster 1985). *N*-acetylation is a major route of metabolism of sulfadimethoxine in catfish (Squibb et al. 1988) and, to a lesser extent, in rainbow trout (Kleinow and Lech 1988).

Renal excretion was a more important route of elimination than biliary excretion. In the biodistribution study, approximately 25% of the intravascular dose (total radioactivity) was eliminated in the urine in 48 h (Table 3). Less than 6% of the dose was found in the bile. The amount of radioactivity contained in the gastrointestinal tract after intravascular dosing was also quite low, suggesting that biliary excretion plays a minor role in the elimination of ^{14}C-ormetoprim. The large percentage of the dose (75%) recovered in the tank water after intravascular dosing indicated extensive elimination of ormetoprim and/or metabolites by an additional route. It is hypothesized that bran-

chial elimination of ormetoprim and nonpolar metabolite occurs. Ormetoprim would be approximately 90% unionized at the pH of fish blood, and highly lipophilic. For polar metabolites, renal excretion would be favored, as apparent by the urinary elimination data in this study (Fig. 3).

In studies of the uptake of ^{14}C-ormetoprim from water, the concentration of the radiolabel (ormetoprim equivalents) in all tissues was less than 0.05 $\mu g \cdot g^{-1}$ and generally below the limit of detection. The bile contained less than 0.1% of the dose at all sampling times. Less than 0.5% of the dose (total radioactivity) was eliminated in the urine in 48 h. Immediately after addition and mixing of ^{14}C-ormetoprim in the six aquaria, the recovery of radiolabel in the tank water was 103 ± 6%, and did not vary over 72 h. The data indicated a negligible uptake of ^{14}C-ormetoprim from water under these conditions.

In summary, ormetoprim disposition in channel catfish is characterized by rapid tissue distribution and extensive tissue penetration. The drug is rapidly absorbed and has a moderate bioavailability. Clearance rates are rapid, although the drug persists in the skin. Ormetoprim is extensively metabolized by catfish. Ormetoprim and/or metabolites are largely eliminated by a route(s) other than biliary or renal.

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Effects of an Experimental Lake Acidification on Zooplankton Feeding Rates and Selectivity[1]

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Although the changes in plankton community composition that result from lake acidification have been documented, little is known about processes that accompany these changes. Here we report investigations on an important process, zooplankton herbivory, in an experimentally acidified lake. Acidification from pH 6.2 to 5.2 has not directly impaired the ability of several major taxa to gather food. Acidification may indirectly affect selective feeding behavior, through changes in the relative abundance of phytoplankton species. Dramatic shifts in population-level grazing were not reflected in overall community herbivory, because of complementary changes in populations in the reference and treatment lake basins. Hence, integrative system-level functions may be poorer indicators of perturbation than specific, fine-scale processes.

Si les modifications provoquées dans la composition de la communauté phytoplanctonique par l'acidification des lacs ont fait l'objet de recherches, on sait peu de chose des processus qui accompagnent ces modifications. Nous faisons ici le point sur un processus important, l'alimentation herbivore du zooplancton, dans un lac acidifié expérimentalement. Le passage d'un pH de 6.2 à un pH de 5.2 n'a pas directement entravé l'aptitude de plusieurs grands taxons à recueillir leur nourriture. L'acidification peut affecter indirectement le comportement alimentaire sélectif par des modifications de l'abondance relative des espèces phytoplanctoniques. Les variations spectaculaires du broutage à l'échelle de la population ne se sont pas reflétées dans la consommation de végétaux de la communauté entière, à cause de changements complémentaires dans les populations des bassins du lac témoin et du lac d'étude. Les fonctions intégrées au niveau du système sont donc peut-être de moins bons indicateurs de la perturbation que les processus spécifiques à petite échelle.

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Distinct changes in planktonic communities occur as a result of lake acidification. Concurrent changes in feeding by aquatic organisms could have important ecological consequences. The role of zooplankton in channelling primary production to higher trophic levels, the potential for zooplankton feeding to affect phytoplankton communities (Porter 1977) and the obvious significance of feeding processes to the health of zooplankton populations make the zooplankton–phytoplankton interface an important place to investigate acidification effects. While changes in plankton community composition and diversity have been described (Almer et al. 1974, 1978; Malley et al. 1982; Roff and Kwiatkowski 1977; Schindler et al. 1985; Sprules 1975; Yan and Stokes 1978), little is known about processes that accompany these changes.

Acidification may affect aquatic organisms either directly, for example by chemical toxicity, or indirectly, through altered interactions with other organisms or the physical environment. Direct pH effects have been cited for the disappearance of some zooplankton species, such as *Mysis relicta* (Nero and Schindler 1983) and *Epischura lacustris* (Malley et al. 1982) and the resultant dominance of acid-tolerant species such as *Diaptomus minutus* (Sprules 1975). The increased abundance of the rotifer *Keratella taurocephala* may be attributable to a combination of acid tolerance and the indirect effects of increased food supply (Yan and Geiling 1985).

There are three ways in which we might expect zooplankton feeding processes to be affected: (1) there may be changes in the feeding rates of individual species; (2) the intensity of overall community herbivory may change; (3) the selective feeding behavior of zooplankton may be affected. Each of these may be caused directly or indirectly by acidification. For example, selectivity may be affected directly, by changes in water chemistry and concomitant changes in the ability of zooplankton to capture particles (Gerritsen and Porter 1982), or indirectly, as a behavioral response to altered phytoplankton composition. Here we examine the effects of acidification on these aspects of zooplankton feeding, by comparing in situ feeding rates and selectivity of major zooplankton taxa in the treatment and reference portions of an experimentally acidified lake. We also attempt to identify the relative importance of direct versus indirect acidification effects on zooplankton herbivory.

Methods

Study Site

Our study area, Little Rock Lake (LRL), Vilas County, WI, is a double-basin, softwater lake with a surface area of approximately 20 ha. It is the site of a whole-lake acidification experiment, in which the north and south basins have been separated with a nylon curtain. The south basin has been

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maintained as a reference, with a pH of approximately 6.2. The north (treatment) basin was maintained at a pH of 5.6 in 1985 and 1986, and then at a pH of 5.2 in 1987. Detailed information on the basic limnology and acidification of Little Rock Lake can be found in Brezonik et al. (1986) and Watras and Frost (1989).

Individual Feeding Rates and Selectivity

We determined in situ feeding rates and feeding selectivity of zooplankton using 7.5-L plexiglass feeding chambers (Haney 1971) and radioisotopically labeled algae. We performed feeding trials in autumn 1986 and intermittently from April through October 1987. We used feeding rates on *Chlamydomonas reinhardtii* (Chlorophyceae, UTEX 90) to estimate generalized feeding rates and to compare those rates in the treatment and reference basins. Because *Chlamydomonas* is extremely rare in both basins of LRL (C. J. Watras, University of Wisconsin, Center for Limnology, unpubl. data) selectivity for it should be free from the effects of strong seasonal changes in its abundance. This should make selection on *Chlamydomonas* fairly consistent, which should improve feeding rate estimates and estimates of selectivity for the other algae.

The algae we used in selectivity trials were *Cryptomonas ozolini* (Cryptophyceae, UTEX LB 2194), *Oocystis marssoni* (Chlorophyceae, UTEX 287, and *Merismopedia* sp. (Cyanophyceae, UTEX LB 1902), chosen from the University of Texas Culture Collection (Starr 1978) for their similarity to dominant phytoplankton species of the same genera in LRL. Because overall feeding rates can vary, we used *Chlamydomonas* as a reference cell with which to compare feeding on other cells (Bogdan and Gilbert 1984). Thus, selectivity for or against one of those three algae is expressed as feeding rate on that alga ("cell B") in relation to feeding rate on *Chlamydomonas*. Details of algal culture and sample analysis will be given elsewhere (M. E. Sierszen, unpubl. data).

To label algae we used only one isotope, ^{14}C, in order to avoid differential isotope loss and because ^{14}C in preserved zooplankton can be retained at nearly 100% efficiency (Sierszen and Watras 1987). We labeled algae with approximately 60 μCi per 25 mL culture, added when the culture was innoculated.

In each feeding trial, we ran paired feeding chambers, one chamber with labeled *Chlamydomonas* and an equal biovolume of unlabeled "cell B," and one with unlabeled *Chlamydomonas* plus labeled "cell B." On several dates we replicated the chamber treatments in order to estimate between-chamber variability in feeding rate measurements. Logistics limited the trials to one "cell B" type per date. We kept concentrations of added cells below $10^3 \cdot mL^{-1}$ in order to minimize effects on natural feeding behavior. After short (10 min) incubations, we retrieved the chambers, sampled chamber water to determine activity, and sieved the zooplankton onto 53-μm Nitex netting. We narcotized the zooplankton in cold carbonated water (Gannon and Gannon 1975) and preserved them by freezing with dry ice, which minimizes the loss of isotopic label that can occur from chemical preservation (Sierszen and Watras 1987). Samples were stored in a self-defrosting freezer, which effectively freeze-dried the zooplankton, pending analysis.

We took one to three subsamples per taxon from each feeding chamber sample, and placed them into scintillation vials. Each subsample contained approximately 50 rotifers, 10 copepods, or five cladocerans. The number of subsamples taken was sometimes limited by the abundance of the taxon in the sample. We placed primary emphasis on analyzing the feeding of three major taxa: the copepod *Diaptomus minutus*, the cladoceran *Holopedium gibberum*, and the rotifer *Keratella taurocephala*. We analyzed additional taxa, listed in Table 1, as abundance allowed, primarily for use in community grazing estimates. We based crustacean feeding rate calculations on standard-size animals. The *Holopedium*, *Daphnia*, and *Diaphanosoma* analyzed were all approximately 0.9 mm long (total length, without tail spine), which was a very common size for these taxa in LRL. We analyzed *Diaptomus minutus* adults and copepodids separately, and did not separate males and females. We determined sample activity by liquid scintillation spectrometry.

Feeding is expressed as clearance rate (millilitres) water cleared of food per animal per hour) and was calculated as ((sample activity/animals per sample)/activity per millilitre chamber water)/0.17 h. We calculated the selectivity coefficient Wi' (Vanderploeg and Scavia 1979) from the median clearance rate of each feeding trial, and tested for significant differences between clearance rates on *Chlamydomonas* and each cell B using Wilcoxon Rank Sum tests (Lehmann 1975). We calculated Wi' by dividing the clearance rate on an item i by the highest clearance rate obtained in that feeding trial. Wi' varies from 0 to 1, with values closer to 0 signifying a greater disparity of clearance rates and a greater degree of selectivity. For graphical clarity, we plotted data from selectivity trials (Fig. 2) as $1\text{-}Wi'$ so that distance from the zero line corresponds to strength of selection.

Community Grazing Rates

To estimate total grazing by the herbivorous zooplankton community, we combined clearance rate estimates for individual taxa with population densities determined in the routine sampling of Little Rock Lake between 1984 and 1987. Because the clearance rates of several major taxa on *Chlamydomonas* appeared to vary seasonally, we regressed median clearance rate obtained on each date against the temperature of LRL at the time and depth of the feeding trial, using simple linear regression (STATGRAPHICS statistical package, STSC, 1986). Temperature was a very good predictor of clearance rate on *Chlamydomonas* for several major taxa. When we obtained a significant ($P<0.05$) linear regression for a taxon, we used that regression to predict clearance rate in LRL. When the regression was not significant, we used the average clearance rate on *Chlamydomonas* from all feeding trials in 1986 and 1987 to estimate the clearance rate of that taxon in LRL. We pooled clearance rates from both basins for these estimates, as there were no significant differences between regressions calculated separately for the treatment and reference basins ($P<0.05$, Tukey-Kramer method, Sokal and Rohlf 1981, p. 507) or, in those taxa for which we used averages, between clearance rates in the two basins (Wilcoxon Rank Sum test, Lehmann 1975).

To calculate population clearance rates, we multiplied the individual daily clearance rate for a taxon by the hypsometrically averaged abundance of that taxon in each basin, determined from routine biweekly sampling with Schindler-Patalas traps from 1984 through 1987 (LRL Database, University of Wisconsin, Center of Limnology). Temperatures used in regressions were also hypsometric averages for each basin, measured during zooplankton sampling. Thus, for each taxon or group we obtain a 4-yr profile of feeding rate, which we can compare among years and between basins. The overall community clearance rate is the sum of these rates on each date,

TABLE 1. Clearance rate regressions and values for major zooplankton groups feeding on *Chlamydomonas*; CR = Clearance rate. (mL·animal^{-1}·h^{-1}; $\overline{CR}$ = average clearance rate; T = temperature; P = regression significance level; F = regression *F*-ratio; n = number of feeding trials.

Taxon	Regression or $\overline{CR}$	P	F	n
Copepoda				
Diaptomus minutus adults	CR = .0229509 + .0213562T	<.001	29.38	28
D. minutus copepodids	CR = −.125039 + .0240237T	.017	15.38	11
Cladocera				
Holopedium gibberum	CR = .25261 + .047127T	<.001	42.23	25
Daphnia spp.	lnCR = −2.51465 + .0847504T	.011	10.16	11
Diaphanosoma birgei	0.48			9
Bosmina + *Eubosmina*	0.068			3
Rotifera				
Keratella taurocephala	lnCR = −4.79981 + .0407328T	.005	9.33	28
K. cochlearis	0.0148			3
K. crassa	0.0159			6
Gastropus stylifer	0.0077			5
Total rotifers	lnCR = −4.96332 + .0407328T			

and is expressed as percent of the water column cleared per day.

To calculate total feeding by the rotifer community, we assumed that feeding rates by all rotifer species followed the same response to temperature as did *Keratella taurocephala*. We averaged the mean clearance rates on *Chlamydomonas* for all rotifer taxa to get an overall average clearance rate. We then adjusted the intercept of the regression equation for *K. taurocephala* so that the regression line would pass through that overall average, and used the adjusted equation to estimate rotifer community grazing.

To test whether there was a change in zooplankton population and community feeding rates in the treatment basin relative to the reference basin following acidification, we used Random Intervention Analysis (RIA, Carpenter et al. 1989). RIA uses paired observations in treatment and reference systems over time to generate a time series of treatment-reference differences, and also calculates mean premanipulation and postmanipulation differences. It then tests for a nonrandom change in intersystem differences following manipulation, using a reference distribution generated by random permutations of all observed differences. If the probability of an observed change in differences is low ($P<0.05$), we can conclude that a nonrandom change has occurred. Such changes may be due to the manipulation, although a significant test result does not necessarily indicate causation.

Results

Individual Feeding Rates

There is no indication that acidification to pH 5.2 has depressed individual feeding rates. We found no significant effect of basin on clearance rates on *Chlamydomonas* for two of the three major taxa (two-way ANOVA; $P=0.20$ for *K. taurocephala*, $P=0.21$ for *D. minutus*). However, clearance rates for *H. gibberum* were significantly higher ($P<0.001$, two-way ANOVA) in the acidified basin than in the reference basin. The average percent difference between median clearance rates on each date ((treatment − reference)/reference) was 24%. Seasonal trends in clearance rates by all three taxa are evident, and are similar in the two basins (Fig. 1, a–c).

Selective Feeding

We found interbasin differences in the selective feeding behavior of two of the three major taxa. The behavior exhibited was different for each of the three algae tested.

On *Cryptomonas*, selection by *K. taurocephala* (Fig. 2a) undergoes a seasonal swing, with selection for *Cryptomonas* relative to *Chlamydomonas* during the summer and early autumn, and selectivity against *Cryptomonas* during late autumn and early spring. This general pattern is similar in both basins; however, statistically significant selection ($P<0.05$, Wilcoxon Rank Sum Test, Lehmann 1975) occurred in the treatment basin only. Selection by *D. minutus* had a similar pattern (Fig. 2b), with less pronounced seasonal variability, but stronger interbasin differences. Preliminary phytoplankton counts reveal summer peaks in *Cryptomonas* populations, coincident with these seasonal selection patterns (T. M. Frost et al., University of Wisconsin, Center for Limnology, unpubl. data). The *Cryptomonas* populations develop in both basins, but much higher concentrations appear in the treatment basin, where almost all of the significant selection for *Cryptomonas* occurred.

For *Oocystis*, there were particularly strong interbasin differences in selection. *D. minutus* (Fig. 2b) exhibited significant selection for *Oocystis* in the treatment basin, but was either nonselective or selected against *Oocystis* in the reference basin. On one date (Fig. 2a), *K. taurocephala* was moderately selective for *Oocystis* in the treatment basin, while exhibiting strong and significant selection against the alga in the reference basin.

For *Merismopedia*, strong interbasin contrasts in selectivity were not apparent. However, interspecific differences can be seen. *D. minutus* (Fig. 2b) never selected against the blue-green, and on one date showed significant selection for it. In comparison, *K. taurocephala* (Fig. 2a) generally had a lower preference for *Merismopedia* than for the reference cell, but never displayed significant selection for or against the blue-green.

H. gibberum was generally less selective than the rotifer or copepod, with $1\text{-}Wi'$ values rarely reaching 0.5 (Fig. 2c). Although significant selection sometimes occurred, strong trends in selectivity were not evident. These results agree with previous studies showing most cladocerans (of Sididae and Daphniidae) to be relatively nonselective feeders (Lampert 1974; DeMott 1982, 1986; DeMott and Kerfoot 1982; Bogdan and Gilbert 1984).

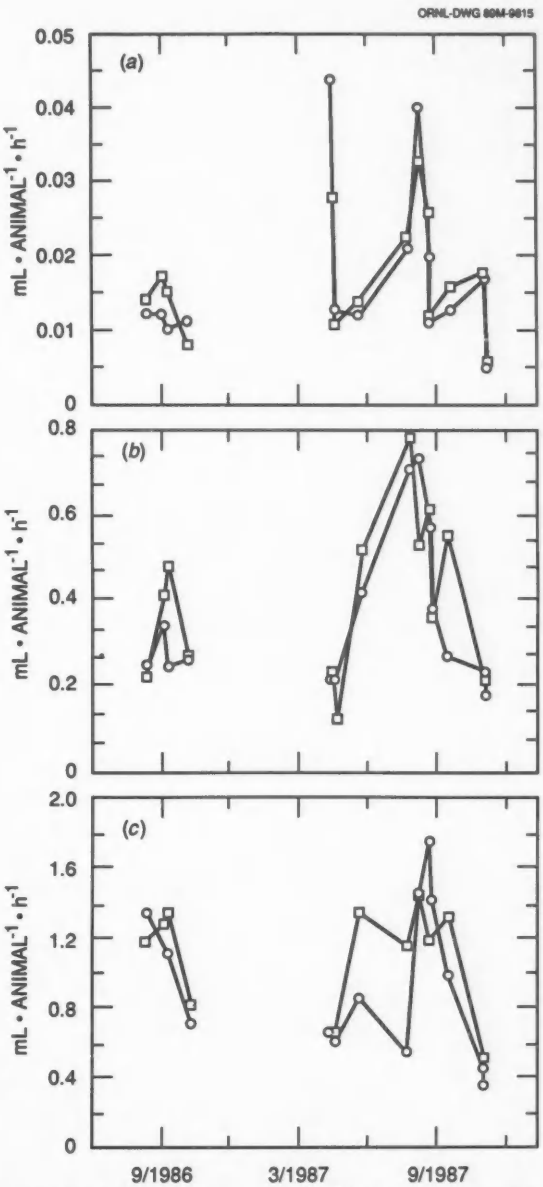

FIG. 1. Clearance rates (mL·animal^{-1}·h^{-1}) of (a) *K. taurocephala*, (b) *D. minutus*, and (c) *H. gibberum* feeding on *Chlamydomonas*, in the reference (circles) and treatment (squares) basins.

Community Grazing Rates

Estimates of total zooplankton community grazing in LRL indicate fairly moderate overall clearance rates. Peak grazing did not exceed 90% of the water column per day in any year except 1986, when it reached 95 and 109%·d^{-1} in the reference and treatment basins, respectively (Fig. 3a). Yearly averages of grazing were on the order of 31–36%·d^{-1}. While similar estimates have been published for other lakes, estimates of mid-summer total grazing in excess of 200%·d^{-1} are not unusual (e.g. Knoechel and Holtby 1986).

Population- and community-level grazing, which we calculated from estimates of individual clearance rates and population densities, incorporate errors associated with those two components. Variability in feeding rate measurements can be estimated from the results of the duplicate feeding chambers run on four dates (Table 2). We used subsamples from each chamber to calculate a median clearance rate per chamber for each of the three major taxa. We then used the medians from duplicate chambers to calculate overall means and coefficients of variation (CV). Variability was generally low between replicate chambers, although the coefficient of variation once reached 0.54 (Table 2, 10/29/87, *K. taurocephala*). An idea of the variability inherent in the population estimates is provided by a spatial heterogeneity study (T. M. Frost, University of Wisconsin, Center for Limnology, unpubl. data). The three major taxa were sampled at 10 stations chosen at random in the acidified basin of LRL on July 2, 1984. The population estimates (animals·L^{-1}, CV in parentheses) are: *D. minutus*, 19.23 (0.39); *K. taurocephala*, 108.10 (0.19); *H. gibberum*, 3.63 (0.61). These population estimates have coefficients of variation which are generally higher than the CV's from the feeding rate measurements (cf. Table 2). Although it would be difficult to combine these two sources of error into a calculation of variance for community grazing rates, the above results suggest that spatial heterogeneity (patchiness) may contribute more error than our estimates of individual feeding rates.

A plot of the series of treatment-reference differences in total grazing (termed BACI, for Before:After, Control:Impact comparison; Stewart-Oaten et al. 1986) shows the differences to be fairly well balanced about zero (Fig. 3b). If total grazing in one basin became higher than in the other, the BACI plot would develop a preponderance of either positive or negative differences. Hence, Fig. 3b indicates no apparent effect of acidification on overall community grazing. Analysis of this time series of differences using RIA (Carpenter et al. 1989) indicates that there was no significant change in total grazing subsequent to the 1985 acidification ($P = 0.435$).

A postacidification increase in the population of *Daphnia* spp. in the treatment basin (Frost and Montz 1988) suggested that *Daphnia* spp. should have exerted substantial grazing pressure there in 1986 and 1987. Accordingly, a BACI plot of estimated total feeding by *Daphnia* (Fig. 4a) shows a clear and highly significant ($P<0.001$, RIA) increase in the treatment basin relative to the reference basin, with differences exceeding 20%·d^{-1}. Because it seemed likely that such a change would have been reflected in the RIA analysis of total grazing, we examined total feeding by the other taxa contributing to the total LRL grazing estimate. Our analyses indicate that the increases in feeding by *Daphnia* spp. in the treatment basin are balanced by comparable increases in feeding by *Holopedium* populations in the reference basin, resulting in no net difference between basins. Total feeding by *Holopedium* populations becomes much higher in the reference basin than in the treatment basin in 1986 and 1987, with interbasin differences of 15 to 20%·d^{-1} (Fig. 4b). This increase is highly significant ($P<0.001$). Analyses of population-level feeding by *D. minutus*, total rotifers, and *D. birgei* revealed no significant changes following acidification, while *Bosmina* + *Eubosmina* showed a significant increase in the reference basin. However, the latter taxa rarely account for more than 15%, and generally average 3%, of community grazing. Further evidence of the counterbalance

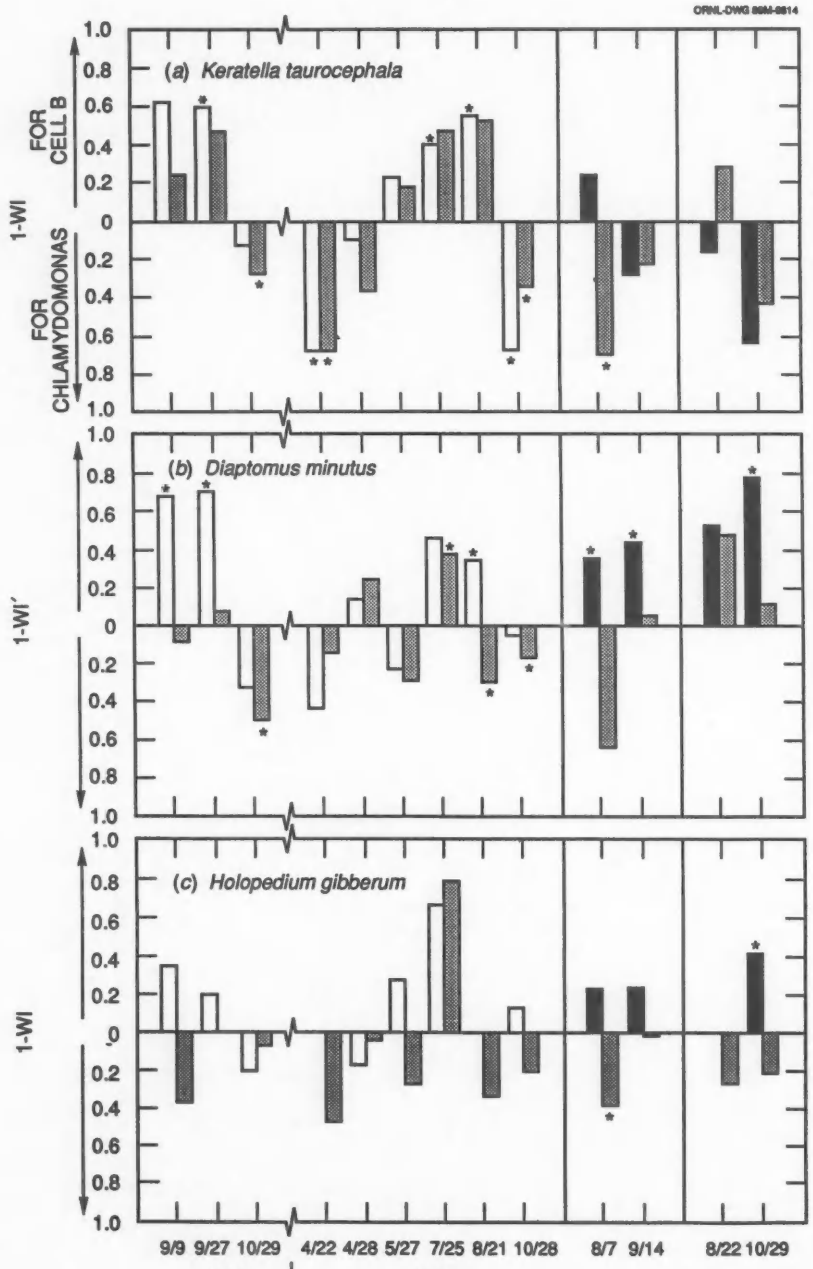

FIG. 2. Selectivity (1-*Wi'*) of (a) *K. taurocephala*, (b) *D. minutus*, and (c) *H. gibberum* for *Cryptomonas* (open bars, left panel), *Oocystis* (dark-stippled bars, middle panel), and *Merismopedia* (solid bars, right panel) with respect to *Chlamydomonas*. These bar patterns refer to selectivity in the treatment basin; lightly-stippled bars to the right of each denote selectivity for same cells in reference basin. Bar length denotes strength of selection; asterisks denote statistically significant ($P<0.05$, Wilcoxon Rank Sum) selection.

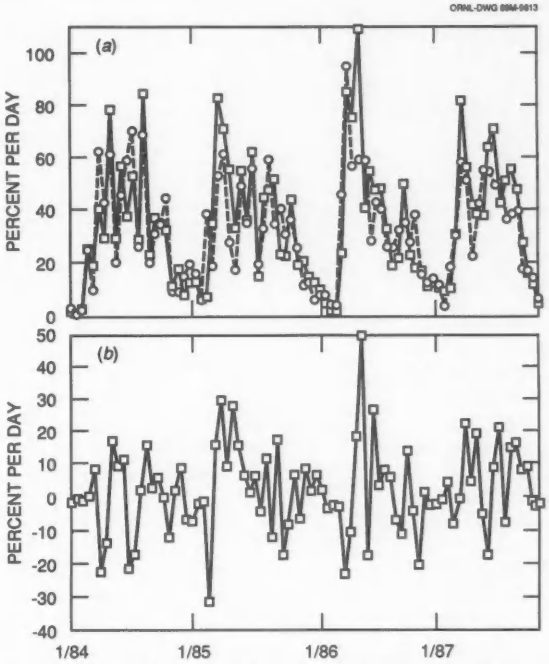

FIG. 3. Total zooplankton herbivory for 1984 through 1987: (a) percent of the water column cleared per day, in the reference (circles) and treatment (squares) basins; (b) BACI plot, time series of treatment-reference differences in total herbivory, 1984 through 1987.

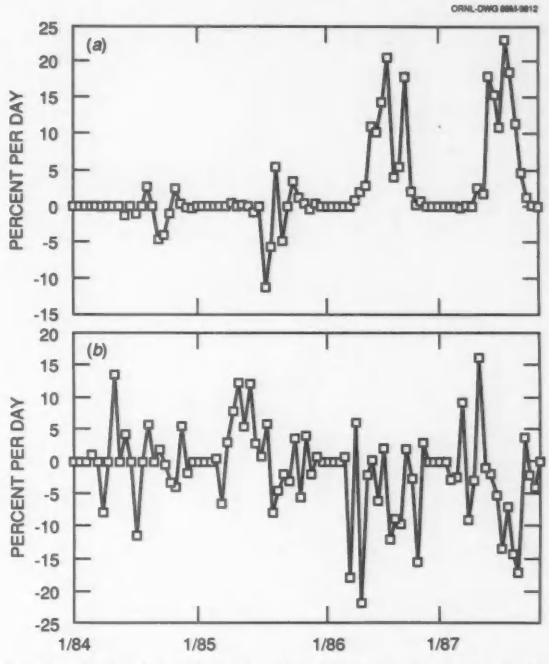

FIG. 4. BACI plots of grazing by (a) *Daphnia* spp. and (b) *H. gibberum*.

TABLE 2. Estimates of variability (mean, CV) in feeding rate estimates for *D. minutus* (D.m.), *K. taurocephala* (K.t.) and *H. gibberum* (H.g.) calculated from replicate chambers run on four dates. ND = no duplicate sample available.

Date	Basin	D.m.	K.t.	H.g.
5/28/87	N	0.49, .23	0.014, .07	1.40, .06
	S	0.42, .03	0.012, .00	ND
8/21/87	N	0.60, .10	0.026, .23	ND
	S	0.56, .11	0.021, .03	1.60, .26
9/15/87	N	0.53, .06	0.016, .06	ND
	S	0.24, .18	0.014, .07	0.99, .08
10/29/87	N	0.21, .05	0.006, .13	0.52, .08
	S	0.17, .06	0.007, .54	0.36, .02

between grazing by *Daphnia* spp. and *Holopedium* is provided by additional RIA analyses. There is no significant interbasin difference in total community grazing after the 1986 increase in *Daphnia* ($P = 0.625$). In contrast, analysis of total grazing without the contribution by *Holopedium* produces a significant ($P = 0.04$) increase in the treatment basin, relative to the reference basin, after the *Daphnia* increase.

RIA analyses detect only relative changes in the reference and treatment systems. When we examine the actual processes behind those relative changes, we find that grazing by *Daphnia* populations not only increased in the treatment basin (from a 1984–85 average of 20.8 $mL \cdot d^{-1}$ to a 1986–87 average of 66.2 $mL \cdot d^{-1}$) but also decreased in the reference basin (from 25.0 $mL \cdot d^{-1}$ to 16.7 $mL \cdot d^{-1}$). Similarly, feeding by *Holopedium* populations not only increased in the reference basin (from a 1984–85 average of 76.1 $mL \cdot d^{-1}$ to an average of 89.6 $mL \cdot d^{-1}$ in 1986–87) but also decreased from 84.1 $mL \cdot d^{-1}$ to 55.7 $mL \cdot d^{-1}$ in the treatment basin. These patterns are evident in Figs. 5a (*Daphnia*) and b (*Holopedium*). Hence, complementary changes occurred in both systems.

Discussion

We found a range of acidification effects on zooplankton feeding in Little Rock Lake. Acidification to pH 5.2 has not had deleterious effects on the ability of *Diaptomus minutus*, *Keratella taurocephala*, or *Holopedium gibberum* individuals to gather food, as measured by feeding rates on *Chlamydomonas*. In fact, acidic conditions appear to have stimulated clearance rates by *Holopedium*. Alternatively, higher clearance rates may be indicative of physiology stress, if, for example, increased metabolic demands have led to higher food intake requirements. Whether *Holopedium* is capable of increasing uptake in response to metabolic demands is not known. Selective feeding behavior differed between basins, among zooplankton species, and among the algae offered. Effects on total community herbivory are not evident, although there were dramatic changes in the overall feeding by important population components of the community. These population-level changes were not driven by changes in the feeding rates of individual animals, but rather by shifts in the abundance of those populations. Because there were commensurate increases in total feeding by different populations in the two basins, differences were not detectable at the community level. Similarly, Bleiwas et al. (1984) found no correlation between total zooplankton grazing and lake pH.

There are several potential sources of error associated with our analyses. First, there may be shortcomings because our

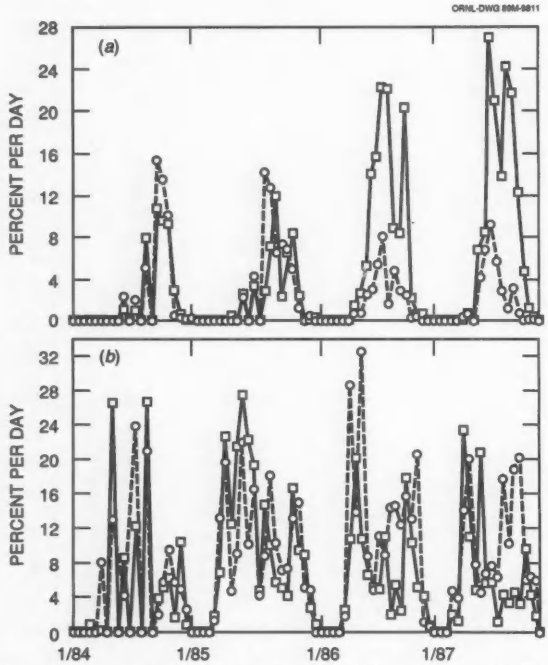

FIG. 5. Total herbivory, 1984 through 1987, by populations of (a) *Daphnia* spp. and (b) *H. gibberum*.

feeding rate estimates were based upon a single cell type, *Chlamydomonas*. However, we feel our approach provides a useful estimate of the grazing behavior of herbivorous zooplankton. The use of several reference cells might give somewhat better feeding rate estimates, but also would be prohibitively complicated and impractical. *Chlamydomonas* serves well as a reference, as it is of manageable size for ingestion (Porter and Orcutt 1980), easily cultured, and, one would expect, free from density-dependent selection in LRL. Another possible shortcoming is that all our feeding trials were performed during daylight hours. Because many zooplankton show increased feeding rates at night (Haney and Hall 1975; Starkweather 1975; Crumpton and Wetzel 1982) our clearance rate measurements may be underestimates. Nonetheless, our estimates of individual and community clearance rates agree well with other published estimates (cf. Bogdan and Gilbert 1984; Bogdan et al. 1980; DeMott 1982 for individual rates; Knoechel and Holtby 1986 and references therein for community rates). Third, in order to make valid comparisons of individual feeding rates, we based our calculations for cladocerans upon standard-sized animals. As a consequence, the actual magnitude of community feeding rates may be somewhat different than those calculated, if there were seasonal changes in body size. The interbasin differences in feeding rates that we present, however, may be unaffected if similar seasonal body-size changes occurred in both basins. We have no evidence of interbasin effects on specific body size. Finally, some errors may have arisen because our estimates of total community grazing were based on measurements of key members of the community, rather than all species, and because we applied our measurements to dates on which grazing rates were not measured. However, we feel that our approach was the best way to combine detailed, seasonal studies of selection with broader estimates of population- and community-level feeding.

At this stage of the acidification of Little Rock Lake, indirect effects on zooplankton feeding processes, through altered interactions with the environment, appear to be much more important than direct chemical effects of increased acidity. Indirect effects are seen in the interbasin differences in selective feeding behavior of some taxa, which appeared to be in response to changes in phytoplankton populations. The changes in population-level total feeding rates were also indirect in nature, caused by changes in the population levels of *Daphnia*, *Holopedium*, and, to a lesser extent, *Bosmina* and *Eubosmina*, rather than by changes in individual feeding rates. In contrast, the higher individual clearance rates by *Holopedium* in the treatment basin may have been a direct response to acid stress. Although this increase was statistically significant, the difference was not strong enough to make the temperature-clearance rate regressions for the two basins significantly different. It is unlikely that the interbasin difference in clearance rates by *Holopedium* were temperature-related, as the treatment basin, where clearance rates were higher, was generally 1–2°C cooler than the reference basin (LRL Database, University of Wisconsin, Center for Limnology).

An interesting aspect of the changes that occurred in the feeding by *Daphnia* spp. and *Holopedium* is that, within each basin, the rise in the feeding by one species was accompanied by a partial decline in the other. Complex processes within each basin that influence the total amount of feeding pressure placed on the phytoplankton may be responsible for this apparent compensation. However, while our data suggest such processes, they have not been conclusively demonstrated, and the apparently complementary dynamics of these species may in fact be independent. Similarly, the relative increase in total feeding by *Daphnia* spp. in the treatment basin and by *Holopedium* in the reference basin may be independent, although invocation of a regulatory process is tempting.

Another crucial point is that none of the observed shifts in total feeding by these populations took place until 1986, a full year after the initial acidification. The existence of a lag time between treatment and effect emphasizes the importance of several years of monitoring after perturbation in order to accurately judge effects.

Schindler et al. (1985) and Schindler (1987) suggest that ecosystem-level functions are poorer indicators of system stress than fine-scale processes. For example, phytoplankton biomass and production did not decrease following experimental perturbations at the Experimental Lakes Area, while there were striking changes in phytoplankton species composition. Our results indicate that this pattern holds for zooplankton-related processes as well. To date, effects of acidification on herbivory in LRL are not detectable at the community level, while population-level responses are dramatic.

D. minutus and *K. taurocephala* are especially tolerant of acidic conditions (Malley et al. 1982). This may be reflected in the lack of direct acid effects on their generalized feeding rates. It is not until we examine a more subtle process, selective feeding behavior, that acidification effects are detected. This underscores the value of analyzing detailed processes, which could be very sensitive indicators of system perturbations.

Lake acidification has many avenues of effect. Direct acid toxicity leading to local species extinction is the most obvious and easily observed phenomenon. More subtle, indirect effects include shifts in relative abundance of both producers and con-

sumers, resulting in changing patterns of resource utilization. Detecting and understanding these subtle but important effects requires detailed analyses of fine-scale processes.

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Structure and Composition of Ice Algal Assemblages from the Gulf of St. Lawrence, Magdalen Islands Area

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De Sève, M. A., and M. J. Dunbar. 1990. Structure and composition of ice algal assemblages from the Gulf of St. Lawrence, Magdalen Islands area. Can. J. Fish. Aquat. Sci. 47: 780–788.

Two types of ice algal assemblages were found in the Gulf of St. Lawrence: assemblages composed predominantly of pennate diatoms (abundance >98.0%) and assemblages with a high abundance of centric diatoms (abundance >46.2%). The first type is similar to Arctic landfast ice algal assemblages with the pennate diatoms *Nitzschia cylindrus*, *N. polaris*, and *Navicula kariana* as dominant species. The second type is similar to drifting ice algal assemblages previously described from the Gulf of St. Lawrence, with a percentage of centric diatom species >46.2% due to the dominance of the planktonic diatom *Thalassiosira nordenskioeldii*. Species richness and the Shannon-Weaver index of diversity were low; density ranged from 10^4–$10^6 \times$ cells·L^{-1} and was negatively correlated with percent centric diatoms. Results on the structure and the composition of the ice algal assemblages are related to ice type, i.e. landfast and drifting pack ice, and compared with ice algal assemblages from higher latitudes.

Deux types d'assemblages d'algues épontiques ont été observés dans le Saint-Laurent : des groupements composés surtout de diatomées pennées (abondance >98,0 %) et groupements présentant une forte abondance de diatomées centriques (abondance >46,2 %). Le premier type est similaire aux assemblages d'algues de la banquise côtière de l'Arctique, les diatomées pennées *Nitzschia cylindrus*, *N. polaris* et *Navicula kariana* constituant les espèces dominantes. Le second type est semblable aux groupements des glaces dérivantes déjà décrits dans le Saint-Laurent, avec un pourcentage de diatomées centriques >46,2 % dû à la dominance de la diatomée planctonique *Thalassiosira nordenskioeldii*. La richesse spécifique et l'indice de la diversité de Shannon-Weaver étaient faibles; la densité allait de 10^{-4} à 10^{-6} cellules par litre et présentait une corrélation négative avec le pourcentage de diatomées centriques. Les résultats concernant la structure et la composition des assemblages d'algues épontiques sont mis en rapport avec le type de glace, banquise ou glace dérivante, et comparés aux groupements d'algues épontiques observés sous de plus hautes latitudes.

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Sea ice algae were first described from the Arctic by Sutherland (1852) and from the Antarctic by Hooker in 1847 (cited in Horner 1985). Early studies of polar ice algae were mainly limited to taxonomic composition (Østrup 1895, 1910; Gran 1904; Usachev 1938, 1949). Pennate diatoms, which belong to the class Bacillariophyceae, are the most commonly occurring and dominant sea ice microalgae. This is particularly true of landfast ice assemblages, in both polar regions, where pennate diatoms account for more than 90% of the species abundance (Burkholder and Mandelli 1965; Meguro et al. 1967; Whittaker 1977; Horner and Schrader 1982; Pett et al. 1983; Poulin et al. 1983; Rymes 1986). According to Dunbar and Acreman (1980), the evolution of such a benthic type algal assemblage was possible because in the Arctic and in the Antarctic the ice forms a more or less permanent upper solid substrate, ecologically comparable with the sea floor.

Centric diatoms have been reported for landfast ice assemblages, but only for a few species and never in great abundance (Grainger and Hsiao 1982). The occurrence of centric species is higher in Antarctic than in Arctic ice, due possibly to the unconsolidated frazil ice structure of Antarctic ice which contains more water between ice crystals, thus providing a more favorable habitat for centric species (Horner 1985). However, centric diatoms are reported as dominant species in a number of studies on drifting pack ice assemblages from both polar regions (Vanhöffen 1893, 1897; Gran 1897; Meister 1930; Usachev 1938, 1949; Hart 1942).

Dunbar and Acreman (1980) found that centric diatoms were dominant in drifting pack ice microalgal assemblages from the Gulf of St. Lawrence, while pennate diatoms were dominant in landfast ice assemblages from higher latitudes. Similar assemblages were described by Demers et al. (1984) from drifting ice floes in the St. Lawrence estuary. The difference in species composition between higher and lower latitudes was attributed to the shorter duration of ice cover and higher under ice light

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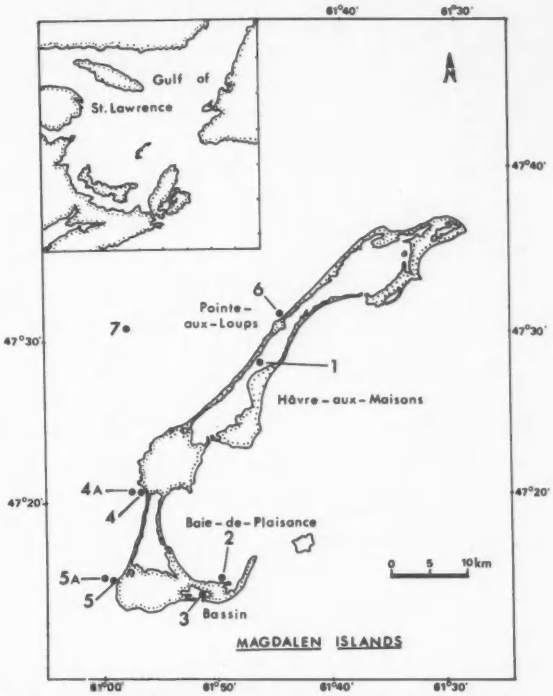

FIG. 1. Magdalen Islands, Gulf of St. Lawrence, Sampling stations are designated by numbers 1 to 6 for landfast ice stations and by number 7 for the drifting ice stations. Stations 4A and 5A are located at the landfast ice edge.

intensity in the Gulf of St. Lawrence (Dunbar and Acreman 1980). Such conditions were suggested to favour planktonic centric diatoms over shade-adapted pennate ones.

This study of ice biota from the Gulf of St. Lawrence was undertaken in view of the results obtained by Dunbar and Acreman (1980) and by Demers et al. (1984). The objective was to determine the structure and composition of ice algal assemblages from landfast and drifting pack ice in the Gulf of St. Lawrence, on a temporal and spatial scale, in comparison with Arctic ice biota and in relation to differences in duration of ice cover and in light regimes. Community structure and species composition were also considered in relation to sea ice characteristics and environmental variables determined previously (De Sève 1989).

Materials and Methods

Study Sites

We sampled six landfast ice stations (1 to 6) and a drifting ice station (7) (Fig. 1). Stations 1 and 3, located in Havre-aux-Maisons and at Bassin, are protected from winds and drifting ice effects. Station 2, located on the eastern coast, is exposed to the northwest winds and to occasional piling up of ice in the Baie de Plaisance. Stations 4 and 5, located on the western side, are exposed to prevailing northwesterly winds and to southeast drifting ice floes coming from the Gulf of St. Lawrence. At these two stations, ice was sampled mid-way between the shore and the edge of the landfast ice (Stations 4 and 5), and at the boundary between landfast ice and drifting pack ice (Stations 4A and 5A) to determine ice edge effects. Station 6, on the northwestern side at Pointe-aux-Loups, is the most exposed to winds and drifting ice. Station 7, located approximately 20 km west of Pointe-aux-Loups, is the drifting pack ice station which was used for comparison to landfast ice.

Sampling and Laboratory Procedures

Landfast ice began to form in the first week of February, but sampling of ice only started at the end of the month when the ice was consolidated and thick enough to work on safely. From that period on, ice samples were collected every 2 wk at Stations 1 to 6. Ice was sampled only twice at Station 7 by helicopter. Ice samples were collected with a SIPRE ice corer of 7.5 cm diameter. A minimum of two cores were collected within a 1-m distance. Ice cores were sectioned (20-cm sections) and numbered by "1" for surface ice down to "B" for bottom ice. The results presented in this paper include the bottom ice section "B" only. In the laboratory, samples were melted in warm water baths (≈16°C). From each sample, two 125-mL aliquots were preserved in acidic Lugol's solution for systematic determination. One aliquot was prepared for identification and cell counts of diatoms using the muffle furnace technique (Zoto et al. 1973). The cleaned frustules were then mounted in Hyrax (Patrick and Reimer 1966) and examined under a Zeiss phase contrast microscope. The second aliquot was analyzed using the Utermöhl (1958) sedimentation technique with a Zeiss phase contrast inverted microscope. This technique is most appropriate for identification and enumeration of flagellates and for evaluation of the percentage of empty diatom frustules.

Data Analysis

A minimum of 500 cells were identified and counted in a series of transects at magnification up to 1250× for subsequent analysis of the structure and composition of the ice algal assemblages. The nomenclature follows VanLandingham (1967–79), and the taxonomic works of Hustedt (1930a,b, 1959, 1966); Cupp (1943); Cleve-Euler (1951, 1952, 1953a,b, 1955); Brunel (1962); and Patrick and Reimer (1966, 1975) were used for the identification of diatoms.

The structure of the various assemblages was analyzed using the following community descriptors:

(1) the number of species, S,

(2) the Shannon-Weaver index of diversity H' (Shannon and Weaver 1949),

(3) Pielou's (1966) evenness index J, defined as the ratio of observed diversity (H') to maximum diversity. When $J = 1$, there is evenness. Departure from 1 is an indication of increasing dominance.

(4) Total density (number of cells·L^{-1}).

(5) Percent centric diatoms, representing the percent contribution in density (cells ·L^{-1}) of centric diatom species over the total density.

The species composition of the various assemblages is determined on the basis of abundance, defined as the percent density (cells·L^{-1}) contribution of individual species over the total density. Dominant species were defined as species with an abundance greater than 5%.

Statistical Analysis

To determine statistical differences in the various community descriptors, in ice among the stations, a SAS statistical package was used in all data analyses (Ray 1982a,b).

TABLE 1. Taxa (Bacillariophyceae and Microflagellates) identified from ice samples in the Magdalen Islands area, including relative abundance (%) in the total collections from bottom ice section (B).

Taxa	B
Bacillariophyceae	
Centrales	
Actinoptychus undulatus Kütz.	0.25
Biddulphia alternans (Bail.)V.H.	0.03
Coscinodiscus asteromphalus Ehr.	0.04
C.decrescens var. *polaris* Grun.	0.38
Cyclotella meneghiniana Kütz.	0.40
C. socialis Schütt	1.23
Ondontella aurita (Lyng.)Ag.	5.35
O.granulata (Roper)Ross	0.09
Paralia sulcata (Ehr.)Cl.	2.09
Thalassiosira decipiens (Grun.)Jörg.	3.48
T. nordenskioeldii Cl.	15.63
Pennales	
Achnanthes haynaldii (Schaarschm)A.Cl.	0.01
Amphora holsatica Hust.	0.24
A.ostrearia (Bréb. ex. Kütz.	0.05
A.perpusilla (Grun.) Grun.	0.04
A.proteus Greg.	0.54
A.proteus var.*oculata* H.& M.Per.	0.01
A.*staurophora* Juhlin-Dannfelt	4.13
Caloneis crassa (Greg)Ross	<0.01
Cocconeis placentula Ehr.	0.38
C.scutellum Ehr.	0.12
C.thomasiana Brun.	0.05
Ctenophora pulchella (Ralfs ex Kütz.)Will. and Round	0.18
Cymbella sp.1	0.05
Diatoma tenue Ag.	0.08
Diploneis incurvata (Greg.)C1.	0.01
D.smithii (Bréb. ex W.Sm)C1.	1.26
D.splendida (Greg.)Cl.	<0.01
D.suborbicularis (Greg.)C1.	1.41
Epithemia adnata (Kütz.)Rab.	0.05
Eunotia lunaris var.*ventosa* (Berg.)Cl.	<0.01
E.pectinalis (O.Müll.)Rab.	0.01
E.vanheurckii Patr.	<0.01
E.veneris (Kütz.)O.Müll.	0.47
Eunotogramma productum (Grun.)V.H.	0.01
Fragilariforma virescens (Ralfs)Will.&Round	0.06
Gomphonema sp.1	<0.01
G.rectum (Donk.)Cl.	0.01
Hantzschia amphioxys (Ehr.)Grun.	0.20
*H.*sp 1.	0.01
Licmophora gracilis var. *anglica* (Kütz.)H.&M.Per.	0.20
Mastogloia angulata Lewis	<0.01
M. apiculata W. Sm.	<0.01
Navicula bacillum Ehr.	0.01
N.cancellata Donk.	3.27
N. cryptocephala var. *veneta* (Kütz.)Rab.	0.01
N. directa (W.Sm.)Ralfs	0.37
N. forcipata Grev.	<0.01
N. galikii (Pant.)Cl.	0.14
N. glacialis (Cl.	<0.01
N. graciloides A.Mayer	0.13
N. grevillei (Ag.)Heib.	1.03
N. humerosa Bréb. ex. W.Sm.	0.17
N. kariana Grun.	20.02
N. laevis A.Cl.	1.00
N. lanceolata (Ag.)Kütz.	0.22
N. lyra Ehr.	5.20
N. muralis Grun.	2.20
N. protracta Grun.	0.20
N. rhyncocephala Kütz.	0.05
N. spicula (Hick.)Cl.	2.26
N. sp.1	<0.01
N. sp.2	<0.01
N. sp.3	0.85
N. yarrensis var.*americana* Cl.	0.91
Nitzschia acuminata (W.Sm.)Grun.	<01.01
N.capitellata var.*lapponica* Hust.	0.15
N.cylindrus (Grun.)Hasle	5.39
N.littoralis Grun.	<0.01
N.polaris Grun.	12.01
N.thermalis Grun.	0.03
N. vitrea Norm.	0.01
Pinnularia baltica Schulz)Cl.-Eul.	0.01
P.globiceps var. *krookii* (grun.)Cl.	3.30
P.quadratarea var. *balticum* Grun.	0.13
P.streptographe Cl.	0.01
Plagiogramma staurophorum (Greg.)Heib.	0.03
Pleurosigma elongatum var. *fallax* (Grun.)Cl.	0.05
Staurosira construens Ehr.	0.09
Staurosirella lapponica (Grun.)Will.&Round	0.01
Surirella islandica Ostr.	0.27
Tabularia investiens (W.Sm.)Will.&Round	0.08
Trachyneis aspera Ehr.	0.03
Microflagellates	1.78

Diversity measures are ineffective in segregating different environments because they do not take into account the species composition of the assemblages compared: thus similarities of species composition and abundance between the different stations were estimated using Ward's agglomerative hierarchical classification (SAS cluster procedure, Ray 1982a). The species density were root–root transformed and used to construct a Bray–Curtis dissimilarity matrix (Bray and Curtis 1957) from which a dendogram is produced, using group-average sorting which joins two groups of sample together at the average level of similarity between all members of one group and all members of the other.

Significant relationships between the various environmental variables and the structure of the assemblages were estimated using Pearson's correlation. To simplify the interpretation of relationships between the environmental variables and the descriptors of the ice algal assemblages, principal component analysis (PCA) procedure (Ray 1982a) was performed. The aim of the PCA was to delineate independent associations among the sets of environmental and community variables. To infer general relationships between the environmental conditions and the structure of the ice algal assemblages, simple linear regression analysis was used to test the relationship between the PC scores of the environmental and algal assemblages data sets.

Results and Discussion

Species Composition

A total of 83 diatom taxa were identified from ice samples (Table 1), with 11 species belonging to the Centrales and 71 species to the Pennales. Microflagellates, the only other group of microalgae present, accounted for a mean abundance of 1.78% in bottom ice (Table 1).

Bottom ice dominant microalgal species differed among stations (Table 2). At Stations 1 and 3, the most abundant species were *Nitzschia polaris* and *Amphora straurophora*. At

TABLE 2. List of dominant diatom species (abundance ≥5%) from bottom ice sections, at Stations 1 to 7, including percent mean abundance, mean density, and standard deviation (in parentheses) per station.

Station	Species	Abundance (%)	Density ($10^3 \times$ cells$\cdot^{-1}$)
1	*Nitzschia polaris*	37	85.9 (±96.5)
	Navicula cancellata	21	48.4 (±71.2)
	Pinnularia globiceps var. *Krookii*	15	35.1 (±32.8)
	Amphora staurophora	7	16.1 (±23.7)
2	*Navicula kariana*	92	359.6 (±425.4)
3	*Nitzschia polaris*	64	157.9 (±201.6)
	Amphora staurophora	17	41.8 (±46.8)
	Navicula muralis	9	21.5 (±25.2)
4	*Navicula kariana*	57	6.1 (±10.8)
	Thalassiosira nordenskioeldii	27	2.9 (±3.1)
4A	*Thalassiosira nordenskioeldii*	46	2.9 (±5.2)
	Navicula kariana	39	3.1 (±4.3)
5	*Thalassiosira nordenskieoldii*	48	3.7 (±5.5)
	Navicula kariana	28	3.6 (±8.4)
	Nitzschia cylindrus	6	0.9 (±8.6)
5A	*Thalassiosira nordenskioeldii*	58	3.4 (±4.8)
	Navicula kariana	34	2.0 (±2.8)
6	*Nitzschia cylindrus*	55	65.7 (±131.4)
	Navicula kariana	37	43.7 (±87.3)
	Thalassiosira nordenskioeldii	37	43.7 (±87.3)
7	*Thalassiosira nordenskioeldii*	36	3.5 (±8.3)
	Nitzschia cylindrus	16	1.4 (±2.2)
	Navicula laevis	11	1.1 (±1.9)

Station 2, the algal assemblage was almost entirely composed of *Navicula kariana* which was also codominant at Station 4, 4A, 5, and 5A with the planktonic species *Thalassiosira nordenskioeldii*. Species composition differed slightly at Station 6 with *Nitzschia cylindrus* being most abundant followed by *N. kariana* and *Thalassiosira nordenskiöldii*. At the drifting ice Station 7, *T. nordenskioeldii* was again dominant with *Nitzschia cylindrus* and *Navicula laevis*.

Species composition of sea ice microalgal assemblages from the Magdalen Islands is similar to assemblages from higher latitudes (Hsiao 1980; Pett et al. 1983; Poulin and Cardinal 1982a,b, 1983; Poulin et al. 1983). *Nitzschia polaris* and *N. cylindrus* are typical cryophiles, reported as dominant species in numerous Arctic ice algal assemblages (Usachev 1949; Hsiao 1979; Horner and Schrader 1981; Pett et al. 1983; Rymes 1986). *Nitzschia cylindrus* is also a planktonic species (Hsiao 1979). *Navicula kariana* is reported from Arctic ice communities, but rarely as a dominant species (Hsiao 1979; Pett et al. 1983; Poulin et al. 1983). *Thalassiosira nordenskioeldii*, a centric diatom with a temperature optimum of 2.3°C (Patrick and Reimer 1966) is a dominant spring phytoplanktonic species in the Gulf of St. Lawrence (Steven 1975) and in the Arctic (Hsiao

TABLE 3. Community descriptors of the bottom ice section (B) at Stations 1 to 7. Descriptors include mean values for *S* (number of species, *H'* (Shannon-Weaver diversity index), *J* (Eveness index), Density (cells$\cdot$L^{-1}, and percent Centric (percentage of centric diatoms). Standard deviations are also included (in parentheses) and number of samples.

Descriptors		Stations								
		1	2	3	4	4A	5	5A	6	7
S	B	20.2	10.4	9.4	6.5	6.0	11.6	4.5	5.5	11.5
		(1.7)	(6.7)	(6.5)	(3.4)	(8.5)	(3.2)	(6.4)	(3.6)	(0.7)
		4	5	5	6	3	5	2	4	2
H'	B	0.74	0.40	0.58	0.42	0.58	0.76	0.43	0.46	0.79
		(0.10)	(0.39)	(0.13)	(0.12)	(0.09)	(0.12)	(0.18)	(0.21)	(0.16)
		4	5	5	6	3	5	2	4	2
J	B	0.57	0.34	0.56	0.63	0.54	0.74	0.45	0.76	0.74
		(0.06)	(0.30)	(0.17)	(0.23)	(0.21)	(0.17)	(0.12)	(0.22)	(0.13)
		4	5	5	6	3	5	2	4	2
Density	B	23.2	48.9	31.0	1.1	1.6	1.4	1.1	12.0	1.0
($\times 10^5$)		(19.2)	(45.1)	(28.9)	(1.6)	(1.3)	(1.7)	(0.9)	(23.7)	(1.3)
		4	5	5	6	3	5	2	4	2
Percent centric	B	1.9	1.3	0.3	29.1	47.1	52.2	61.8	5.0	40.8
		(1.9)	(2.1)	(0.1)	(17.3)	(10.2)	(5.2)	(9.1)	(12.5)	(19.4)
		4	5	5	6	3	6	2	4	2

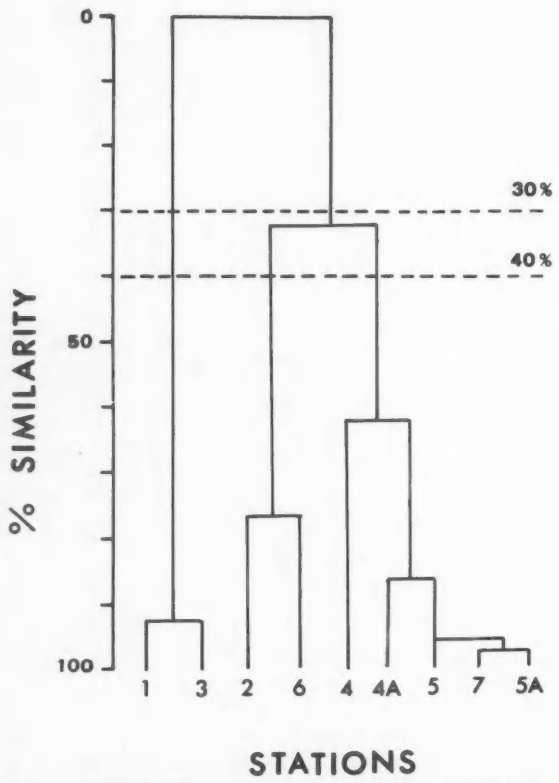

FIG. 2. Dendogram showing classification of Stations 1 to 7 based on species composition, i.e. mean species density (No. of cells·L^{-1}). Stations split into two main groups at the 30% similarity level, and the second group splits into two main subgroups at the 40% similarity level.

1983). It is also found in association with Arctic ice algal assemblages (Alexander et al. 1974; Pett et al. 1983; Rymes 1986), but never as a dominant species. In the Magdalen Islands, it accounted for the high percentage of centric diatoms. Dunbar and Acreman (1980) and Demers et al. (1984) also found *T. nordenskioeldii* as a dominant species, along with other centric diatoms.

Structure of Ice Algal Assemblages

Mean number of species ranged from 20.2 at Station 1 to 4.5 at Station 5A (Table 3). The number of species at Station 1 differs significantly from that at Stations 4, 4A, 5A, and 6 (ANOVA, Student-Newman-Keuls test, $p<0.05$). The Shannon-Weaver diversity indices (H') were low (Table 3) but did not differ significantly between stations (ANOVA). Evenness index varied from 0.76 at Station 6 to 0.34 at Station 2, but did not differ significantly between stations (ANOVA). Departure of J from 1, an indication of dominance, occurred particularly at stations with high percent abundance of dominant species such as Station 2 where *Navicula kariana* contributed 92% of the abundance. Density was quite variable ranging from a minimum mean value of 0.97×10^5 cells·L^{-1} at Station 7 to a maximum of 48.9×10^5 cells·L^{-1} at Station 2 (Table 3). Cell numbers were an order of magnitude higher at Stations 1, 2, 3, and 6 (10^6 cells·L^{-1}) than at Stations 4, 4A, 5, 5A, and 7 (10^5 cells·L^{-1}). Despite such differences in mean density between stations, they were not significantly different (ANOVA) because of the very high standard deviations associated with cell density. The results on percent centric species indicated a clear demarcation in structure between stations (Table 3) with statistically significant differences (Student-Newman-Keuls test, $p<0.05$). At Stations 1, 2, and 3 located in lagoons and on the eastern coast of the Islands, the assemblages were composed of a majority of pennate species, with centric diatoms contributing to less than 2% of the abundance. The assemblages from Stations 4, 4A, 5, and 5A, located on the western coast and from the drifting ice Station 7 had a different composition, with centric species accounting for 29% or more of the abundance. These assemblages correspond to the two major groups of stations recognized on the basis of the dendogram (Fig. 2) produced from Ward's agglomerative hierarchical classification.

Species richness and diversity values of bottom ice algal assemblages found in this study, were much lower than those of ice algal assemblages from higher latitudes: Rymes (1986) reported a species richness of $S=47.7$ and diversity values of $H'=1.14$ for Baffin Island. Considering the ice substrate as an energetic interface or "ergocline" (Legendre and Demers 1985) similar to a benthic substrate, comparison of the two environments can be made. Diversity of undisturbed benthic diatom assemblages is in the average of $H'=1.40$ (O'Quinn and Sullivan 1983), but can be reduced to less than 0.70 in polluted or turbulent areas (De Sève and Goldstein 1981; Luttenton and Rada 1986). Species diversity of $H'=1.09$ and less are considered as typical of unstable environments (Margalef 1978). In the Gulf of St. Lawrence, the instability of the ice substrate, due to melting and rafting of landfast and drifting pack ice (De Sève and Dunbar 1990a) could account for the low diversity observed. Further, short duration of ice coverage (less then 2 mo) might be responsible for low species richness typical of diatom assemblages in early stages of colonization (Hudon and Bourget 1981).

Density of bottom ice assemblages in the Gulf was one to two orders of magnitude less than that of Arctic landfast ice communities, with mean values of 13.5×10^4 cells·L^{-1} compared with density values of $>10^7$ cells·L^{-1} at higher latitudes (Grainger and Hsiao 1982; Pett et al. 1983; Poulin et al. 1983; Rymes 1986), but in the same range as those reported from drifting pack ice in the St. Lawrence estuary (Demers et al. 1984). Density was also negatively correlated with percent centric diatoms, with cell per litre one order of magnitude higher at stations with a low percent abundance of centric diatoms. Nutrient limitation may explain reduced ice algal biomass in the Gulf of St. Lawrence (De Sève and Dunbar 1990b) but lack of correlation between the two, coupled with evidence of heterotropic uptake of organic substances by ice diatoms (Rivkin and Putt 1987) does not lend strong support to this hypothesis. Low density might result from the instability and short duration of the ice substrate, two factors which can limit growth and algal biomass accumulation.

On the basis of percent centric diatoms, two types of ice algal assemblages were described from the Magdalen Islands area. The first type is almost entirely composed of pennate diatoms, with centric species contributing less than 2% of the abundance. This type of community is similar to landfast ice algal assemblages from higher latitudes (Horner 1985). It is the first time that such assemblages are described for the Gulf of St. Lawrence. The second type of community is composed of a high

TABLE 4. Eigenvector loadings and Pearson's correlation coefficients (r) for correlations between descriptors and principal components (PC) 1, 2, and 3; statistical significance (two-tailed test) is indicated by *** at $p<.002$, ** at $p<.01$, * at $p<.02$ and + at $p<.05$; $n=9$ stations.

Community descriptors[a]	PC1		PC2		PC3	
	Loading	r	Loading	r	Loading	r
S	0.389	0.50	0.512	0.63	0.413	0.12
H'	−0.021	−0.05	0.699	0.92***	0.215	−0.03
J	−0.260	−0.12	0.486	0.67+	−0.775	−0.65
Density	0.630	0.88***	−0.113	−0.22	−0.195	0
Percent centric	−0.619	−0.93***	−0.021	−0.15	0.380	0.32

[a]Abbreviations used: S = species richness; H' = Shannon-Weaver diversity index; J = Pielou's evenness index; Density = cells·L^{-1}; Percent centric = percent abundance of centric diatom species.

percentage of centric diatoms (29.1 to 61.8%) and is similar to the assemblages described by Dunbar and Acreman (1980) and Demers et al. (1984) from drifting pack ice in the Gulf and the St. Lawrence estuary. Dunbar and Acreman (1980) and Demers et al. (1984) attributed the differences in systematic composition of ice flora between the Gulf and higher latitude assemblages to (1) the short duration of ice cover in the Gulf (2 mo), and (2) to higher underice light levels in the Gulf due to thinner ice and increased light intensity from lower latitudes, favouring the growth of planktonic centric diatom over shade adapted pennate forms. With respect to systematic composition of the first community type, the results of this study do not lend support to Dunbar and Acreman's and Demers' et al. hypothesis since ice algal assemblages similar to Arctic landfast ice assemblages were found in the Gulf of St. Lawrence. Further, their implication of higher underice light levels favouring the growth of centric diatoms over pennate forms is not substantiated in this study since assemblages with a high percentage of centric species had densities one order of magnitude less than assemblages with pennate diatoms as dominant ones. But higher underice light intensities in the Gulf: 100–200 $\mu Ein \cdot m^{-2} \cdot s^{-1}$ (De Sève 1989), compared with 1–2 $\mu Ein \cdot m^{-2} \cdot s^{-1}$ during Arctic ice algal bloom (Grainger 1977) could account for the lower density and biomass of the Gulf assemblages by not allowing for the extreme shade–adaptation typical of Arctic ice algae (Cota et al. 1987).

Structure of Ice Algal Assemblages and Environmental Interactions

In the principal component analysis (PCA) performed on the descriptors of the algal assemblages, PC1 accounted for 46.6% of the variance, with a high positive loading on density and percent centric (Table 4, $p<0.002$). PC2 accounted for 37.8% of the variance with high positive loading on H' at the $p<0.002$ level. Therefore, major differences in the structure of the ice algal assemblages were primarily related to density and percent centric diatoms. These two descriptors were negatively correlated between each other (Pearson's correlation coefficient $r=-0.83$, $p<0.005$) with highest densities found at stations where abundance of percent centric diatom was low, namely Stations 1, 2, and 3. From the three-dimensional projection onto the PC1, PC2, and PC3 of the PCOM (Fig. 3), the stations are again grouped similarly as in the dendogram produced in Fig. 2 from species composition clustering analysis.

Principal component analysis (Table 5) for environmental variables measured during this study (De Sève and Dunbar 1990b) revealed that PC1, which accounted for 36.7% of the

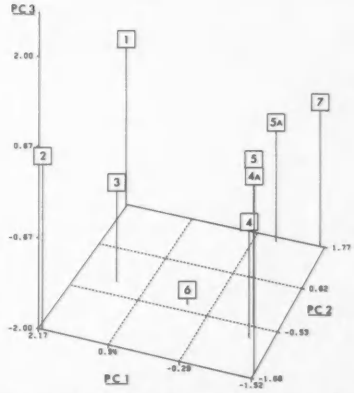

FIG. 3. Three-dimensional projection of Stations 1 to 7 onto the first three principal components (PC1, PC2, and PC3) of principal component analysis based on a matrix of correlations among community descriptors (PCOM) for bottom ice sections.

variance in the data, had a high positive loading on silicates and a high negative loading on nitrates, both significant at $p<0.002$ level. Positive loading was also observed for chlorophyll *a*, but only at the $p<0.05$ level. PC2 accounted for 27.7% of the variance, and had a high positive loading on orthophosphates and phaeopigments, significant respectively at $p<0.002$ and $p<0.01$. Because PC1 and PC2 together accounted for 64.4% of the variance, major differences among the stations were related to nutrients, namely nitrates, silicates, and orthophosphates.

To assess any relationship between the descriptors of the ice algal assemblages and the environmental variables, Pearson's correlations were performed between the three principal components of the environmental variables and the community descriptors. The results (Table 6) indicate a lack of correlation between community descriptors and PC1, PC2, and PC3 of the environmental variables, suggesting that factors other than nutrients are responsible for the variations in density and percent centric diatoms observed between the stations.

Principal component analysis relating environmental variables to the structure of the ice algal assemblages failed to show any significant correlations that could account for the existence of the two types of ice biota communities. However, a relationship was found between the community types and the location of the stations. Assemblages with a dominance of pennate diatoms were found in lagoons and on the eastern coast of the Islands, at landfast ice stations protected from winds and drift-

TABLE 5. Eigenvector loadings and Pearson's correlation coefficients (r) for correlations between environmental variables and principal components (PC) 1, 2, and 3; statistical significance (two-tailed test) is indicated by *** at $p<.002$, ** at $p<.01$, * at $p<.02$ and + at $p<.05$; $n=9$ stations.

Environmental variables	PC1		PC2		PC3	
	Loading	r	Loading	r	Loading	r
Salinity (‰)	0.089	0.14	−0.406	−0.56	0.618	0.70+
PO_4-P (μM)	0.032	0.05	0.674	0.94***	0.170	0.19
NO_3-O (μM)	−0.506	−0.81***	0.052	0.07	−0.066	−0.07
NO_2-N (μM)	0.395	0.63	−0.177	−0.25	−0.514	−0.58
Si $(OH)_4$ (μM)	0.541	0.87***	−0.186	−0.26	−0.187	−0.21
Chlorophyll *a* ($mg \cdot m^{-3}$)	0.417	0.67+	0.064	0.09	0.533	0.60
Phaeopigments ($mg \cdot m_{-3}$)	0.333	0.53	0.555	0.77**	−0.037	−0.04

TABLE 6. Pearson's correlation coefficients between three principal components (PC1, PC2, and PC3) of environmental variables and community descriptors; no statistical significance was observed.

Community descriptors[a]	PC1	PC2	PC3
S	0.03	−0.28	−0.55
H'	−0.19	−0.23	−0.64
J	−0.48	−0.47	−0.11
Density	0.04	0.26	−0.23
Percent centric	0.05	−0.16	0.32

[a]Abbreviations used; S = species richness; H' = Shannon-Weaver diversity index; J = Pielou's evenness index; Density = number of cells·L^{-1}; percent centric = percent abundance of centric diatom species.

ing pack ice. Such stable landfast ice conditions would favour the establishment of assemblages similar to higher latitude landfast ice communities. Assemblages with a high percentage of centric species were found at the drifting ice station (Station 7) and at stations located on the western coast of the Islands (Stations 4, 4A, 5, and 5A). Similarity of species composition at these stations with the drifting pack ice assemblages described by Dunbar and Acreman (1980) and Demers et al. (1984), for the Gulf and the St. Lawrence estuary suggests a correspondence in species composition with ice types. This is particularly true considering that the landfast ice stations located on the western coast of the Magdalen Islands are subjected to the influence of drifting pack ice from the estuary and the Gulf of St. Lawrence by (1) being directly in the line of flow of the drifting pack ice and (2) being directly exposed to the prevailing northwesterly winds which cause rafting of drifting pack ice under landfast ice (De Sève and Dunbar 1990a).

Furthermore, the occurrence of centric diatoms is rare for landfast ice assemblages (Grainer and Hsiao 1982; Pett et al. 1983) but there are numerous reports of ice algal assemblages from drifting pack ice where centric diatoms are dominant (Vanhöffen 1893, 1897; Gran 1897; Meister 1930; Usachev 1949; Mel'nikov 1980). Evidence for the existence of two floras on the bottom of Arctic sea ice, distinguishable in species composition and related to ice of different origin, was even put forward by Apollonio (1985) in his account of Sutherland's (1852) report of a drifting pack ice community, off the west coast of Greenland, dominated by centric diatoms.

Our results substantiate the existence of the two flora and their relationship to ice types, but the mechanisms involved are still a matter for speculation. The abundance of pennate diatoms in landfast ice algal assemblages was thought to result from the close proximity of landfast ice to the benthos, from which it could be seeded by benthic pennate diatoms. There is however no significant species correlation between the benthic and ice diatom flora (Horner 1985), except of the dominance of pennate forms. Indeed, the majority of widely distributed and dominant ice diatoms are typical cryophiles found only in association with ice (Usachev 1949). Pack ice formed in deep waters (>200 m), away from coastal influence, is expected to contain more phytoplanktonic centric diatoms than pennate diatoms, since they are the dominant species present in the water column. Actually, the co-occurrence of phytoplanktonic species in drifting pack ice and in seawater has led to the hypothesis that drifting pack ice may be a seasonal habitat for planktonic forms (Garisson and Buck 1985; Garrison et al. 1987). In the Gulf of St. Lawrence, the abundance of *Thalassiosira nordenskioeldii* in drifting pack ice and as a spring phytoplankton species would appear to support this hypothesis.

Differences in the life cycle of pennate versus centric diatoms could partly account for the existence of the two floral types. Many centric species form resting spores (Hargraves and French 1983), including *Thalassiosira* sp. (Fryxell et al. 1979; Durbin 1978), but this rarely occurs in pennate forms (Von Stosch and Fecher 1979). The absence of resting spore formation in pennate ice diatoms could limit their survival to coastal shallow areas, but future research is needed, particularly with respect to the life cycle of pennate versus centric ice diatoms, to fully understand the relationships between ice types and the different ice flora observed.

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Use of Artificial Substrates to Estimate Prey Resources available to a Visually Feeding Benthivorous Fish

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Empirically derived estimates of prey resources using conventional sampling methods are generally poor indices of actual food available to visually feeding benthivorous fish. We preferentially sampled the most active fraction of the benthos (presumably that most detectable by fish) utilizing short-term (4 d) colonization of artificial substrates, and used colonist biomass to explain temporal variation in stomach contents of bluegill sunfish (*Lepomis macrochirus*) in Lake Opinicon, Ontario. Simple linear regressions showed that total biomass of colonists explained 75% of the variance in the biomass of stomach contents. Independent variables using a limited taxonomic range or size range of colonists explained less variance in stomach contents. The convenience and simplicity of this methodology, and its ability to explain within-lake variation in food exploitation by visual benthivores, suggest that artificial substrate colonization could be useful for surveys of prey availability to benthivorous fishes.

Les évaluations du bassin de proies, calculées empiriquement selon les méthodes classiques d'échantillonnage, constituent généralement de mauvais indices de l'abondance des proies accessibles aux poissons benthivores qui se nourissent à vue. Nous avons échantillonné préférentiellement la fraction la plus active du benthos (présumément celle qui est le plus discernable par les poissons) par colonisation à court terme (4 d) de substrats artificiels; nous avons utilisé la biomasse des colonisateurs pour expliquer les variations temporelles observées dans les contenus stomacaux de Crapets arlequins (*Lepomis macrochirus*) du lac Opinicon en Ontario. Par régression linéaire simple, nous avons montré que la biomasse totale des colonisateurs permettait d'expliquer 75 % de la variance observée lors de l'analyse des contenus stomacaux. La commodité et la simplicité de cette méthodologie, alliées à son potentiel d'expliquer les variations intra-lac de l'exploitation des proies par les benthivores qui chassent à vue, indiquent que la colonisation de substrats artificiels pourrait se révéler utile aux relevés de l'accessibilité des proies aux poissons benthiques.

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Conventional techniques for sampling benthic invertebrates are costly (Soszka 1975; Downing 1984, 1986; Konkle 1988). They are inefficient methods for sampling the benthic resource available to benthivorous fish because they fail to distinguish between total benthos and that small portion actually available to visually feeding benthivores (Boisclair and Leggett 1985). Limitations of current benthic sampling methods and the potential benefits from improving measures of available benthos are suggested by results of Hanson and Leggett (1982) who identified lake average benthic biomass values and total phosphorus (P) concentration as the best predictors of fish biomass or yield in north-temperate lakes. Total P was a more consistent predicator of fish variables than measures of benthic biomass (in terms of variance explained in both simple and multiple regressions), even though the effects of P are two trophic levels further removed from piscivores than benthic biomass. It seems probable that the predictions derived from benthic biomass were degraded by emphasis on profundal over littoral sampling, and the fact that only a small portion of total biomass is available to fish. Development of a convenient measure of available benthos should therefore improve our ability to predict fish production in lakes, and facilitate basic understanding of fish–benthos interactions.

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Here we use a new method for sampling littoral macroinvertebrates, based on short-term colonization of artificial substrates (Konkle 1988), to estimate the prey resources potentially available to visually-feeding benthivorous fishes. Colonization of artificial macrophytes reflects seasonal changes in the littoral macroinvertebrate community, and short colonization times select for the most active organisms, those most likely to be detected by visual predators (Macan 1977; Konkle 1988). In this paper, measures of artificial substrate colonization are used in linear regression analyses to explain variation in food intake of a widespread, visual benthivore, the bluegill sunfish (*Lepomis macrochirus*; Gerking 1962; Scott and Crossman 1973; Carlander 1977; Werner and Hall 1979).

Methods

The study was conducted in Lake Opinicon, Ontario, during the summers of 1984 and 1985. The littoral zone of this lake supports large resident populations of several widespread littoral fish species, with bluegill dominating in terms of abundance and biomass through most of the summer (Keast and Harker 1977; Keast 1978a,b, 1980). Keast et al. (1978) and Konkle (1988) describe the lake and the study sites.

Each artificial macrophyte was made of 20 strips (90 × 2 cm) of green polyethylene plastic cut from garbage bags that

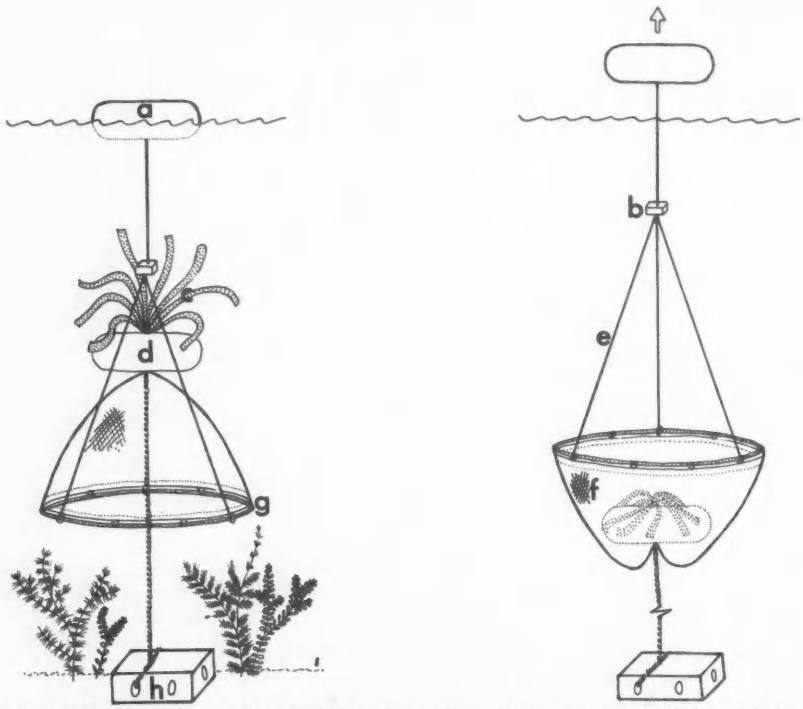

FIG. 1. Artificial substrate used to sample littoral macroinvertebrates, shown in situ (left) and during retrieval (right). a = surface float, b = wood float, c = plastic leaf mass, d = float, e = retrieval line, f = retrieval net, g = aluminum ring, h = brick anchor.

were tied with wire at the centre of the strips to form a "leaf mass" with a total surface area of 3600 cm^2 (Fig. 1). A 1.5-m length of 7-mm diameter three-stranded green polypropylene line, anchored to the bottom by a brick, served as the stem. Stem height could be adjusted by tying the anchor at different lengths of the line. The leaf mass was attached to the top of the stem with wire and kept afloat with a green styrofoam float 9 cm in diameter and 7 cm high. A retrieval net made from a cone of 250-μm Nitex mesh was attached to a 30-cm diameter aluminum ring, and the apex of the cone was attached to the stem just below the leaf mass. This net enclosed the substrate during retrieval, preventing loss of invertebrates from the leaf mass. Three 80-cm long lines (5 mm diameter) extended from the aluminum ring of the net to a small wooden float above the leaf mass. A surface float was attached to these lines by an aluminum clip. Substrates could be easily deployed and retrieved from a boat. Height of the substrate above the sediments was adjusted periodically to keep the leaf mass even with the upper two-thirds of the natural macrophytes.

Ten artificial macrophytes were deployed among natural littoral macrophytes for 4-d exposure periods on 10 dates during June and August, the period of maximum macrophyte and fish growth (Keast 1978b; Booth 1984). Substrates were subsequently retrieved, transported to the lab, and washed in 2% ethanol to remove all macroinvertebrates. Invertebrates were concentrated by filtering the sample through a 250-μm sieve, and were preserved in 70% ethanol. Concurrently, and at the same sites, age II (55–75 mm fork length; FL) and age III (76–102 mm FL) bluegill (N = 254 total) were collected by purse seine, trapnet, or fish traps (Kushneriuk and Paloheimo 1984) between 0800 and 1100, which corresponds to the peak feeding period for bluegill in Lake Opinicon (Keast and Welsh 1968; Booth 1984). Fish were anaesthetized, measured (FL in millimetres), and stomach contents were removed by pulsed gastric lavage (Foster 1977) with filtered lake water. Stomach contents were concentrated with a 153-μm mesh sieve and preserved in 70% ethanol.

Macroinvertebrates from both artificial substrates and stomachs were identified, enumerated, and measured (total body length exclusive of caudal appendages) using a microcomputer-based caliper and video system (Sprules et al. 1981). Dry mass of colonists (milligrams DW) was determined from length–mass conversion formulae from the literature (Konkle 1988).

Simple linear regression analysis (Zar 1984) was used to relate average biomass of stomach contents to the following measures of substrate colonization: (1) mean total invertebrate biomass per substrate; (2) mean biomass of active taxa, (i.e. those most likely to have colonized substrates by swimming, or which were known to be active in the water surrounding natural macrophytes (Soszka 1975); they included zooplankton, amphipods, ostracods, chironomids, mites, mayflies, and trichopterans, and excluded odonates, gastropods, isopods, and leeches); and (3) mean biomass of invertebrates <0.5 mg DW, which included >90% of total colonist biomass. All regressions included 10 datapoints. Sample sizes for substrates and fish collections are given in Table 1.

Our measure of fish food intake was also refined to see if the unexplained variance could be reduced. In one refinement the

TABLE 1. Biomass (and SE) of macroinvertebrates colonizing artificial substrates (A.S.) during 4-d exposure periods in Lake Opinicon, and biomass (and SE) of stomach contents from fish caught concurrently at the same sites. Dates indicate day on which A.S. were removed from the lake. WPB = White Pine Bay, SHI = Sheep Island, LBB = Long Beach Bay. Sample sizes (*N*) are given for fish and A.S. samples. Seasonal means and standard errors (SE) are also given.

Date	Site	*N* A.S.	*N* Fish	Mean colonist Biomass (mg DW)		Mean stomach Contents (mg DW)	
June 18, 1984	WPB	10	20	49.61	(6.84)	0.93	(0.64)
June 26, 1984	WPB	8	24	27.22	(7.57)	3.96	(1.15)
June 26, 1984	SHI	10	20	48.29	(6.49)	5.88	(1.80)
July 9, 1984	WPB	10	22	28.04	(4.09)	0.45	(0.09)
July 20, 1984	WPB	10	20	26.51	(5.52)	4.88	(1.06)
August 10, 1984	WPB	9	13	10.80	(1.59)	0.32	(0.15)
August 10, 1984	LBB	10	20	11.19	(2.04)	3.32	(1.11)
Season mean				28.81		2.82	
SE				5.45		0.79	
June 16, 1985	WPB	10	20	38.54	(9.09)	0.88	(0.34)
July 15, 1985	WPB	10	20	15.40	(3.66)	0.87	(0.24)
July 24, 1985	WPB	10	20	9.37	(1.04)	0.51	(0.46)
Season mean				21.10		0.75	
SE				7.26		0.10	

two age/size classes of fish were separated and examined individually. In another refinement, stomach contents were adjusted for fish size by dividing biomass of stomach contents by fish mass. Fish wet mass (W) was determined from the following length–mass conversion formula developed from measurements of 244 Lake Opinicon bluegill: W (grams) = 1.412E-06 $TL^{3.534}$, r^2 = 0.98, where TL (millimetres) = 0.8155 FL.

Results

Individual and Seasonal Variation in Food Consumption

The range and mean of the coefficient of variation for average mass of stomach contents were 96–179% and 136%, respectively. These values are much higher than corresponding estimates for average colonist biomass on substrates (range of 26–74%, mean of 53%; Konkle 1988), suggesting that the sample sizes necessary to estimate stomach contents need to be much higher than sample sizes of artificial substrates.

Regression Analysis

Measures of artificial substrate colonization were generally good indices of bluegill food consumption, explaining much of the variation in stomach contents in some cases (Figs. 2, 3). The best indicator of food intake for both fish size-classes combined was total colonist biomass, which explained 75% of the variation in total biomass of stomach contents. Total colonist biomass also explained a relatively large portion of the variability in stomach contents of age II fish (r^2 = 0.73). All refinements of both independent variables (artificial substrate measures) and dependent variables (stomach contents), made to determine whether the variance in food intake could be more completely accounted for by artificial substrate colonization, failed to improve the amount of variance explained by total colonist biomass.

Discussion

Measures of the short-term colonization of artificial macrophytes appear to be relatively good indicators of food exploitation by benthivorous fish. This strong relationship between bluegill foraging and food availability may reflect the facts that bluegills tend to feed on active prey (Werner and Hall 1974; Vinyard 1980) and that, due to the short colonization periods, colonists of artificial macrophytes will tend to be the more active organisms. The positive relationship between available and/or energetically profitable prey resources and fish foraging or growth, and the potential for improving predictions of foraging success with better measures of prey availability, have been recognized by several researchers (Ball 1948; Gerking 1962; Werner and Hall 1979; Mittelbach 1981b, 1988; Boisclair and Leggett 1985).

The failure of all the regression refinements to improve the variance in fish stomach contents explained by total colonist biomass was somewhat surprising. Each refinement, based on biological constraints, represents an a posteriori attempt to reduce random variation in stomach contents or substrate colonization. The first refinement, restricting colonist biomass to a limited subset of active taxa which contributed to the bulk of colonist biomass, excluded some large and mobile organisms such as odonates, which were rare on substrates and in fish stomachs. These prey items could have represented a significant portion of the diet of bluegill during some sampling periods, and apparently explained a substantial part of the variance in stomach contents. Similarly, the second refinement eliminated all organisms >0.5 mg DW from the analysis and apparently removed similar information about rare food items.

Controlling for variation in fish foraging associated with differences in fish size also failed to improve the variance explained; it yielded the poorest relationship of all regressions. However, this result should not be taken as evidence that fish size can be ignored in future studies relating fish resources to foraging success. We suggest, rather, that the failure to improve explained variance by taking into account fish size is an artifact of the rather narrow size range of fish we studied. Studies of bluegill foraging in Michigan ponds (Mittelbach 1981a,b; Werner and Hall 1979; Werner et al. 1983) and Lake Opinicon (Keast 1978a,b, 1980) show that there is little difference in feeding rate or diet of fish in the size range studied here. Therefore, the refinement may have simply reduced sample size for each regression datum and thereby increased variance.

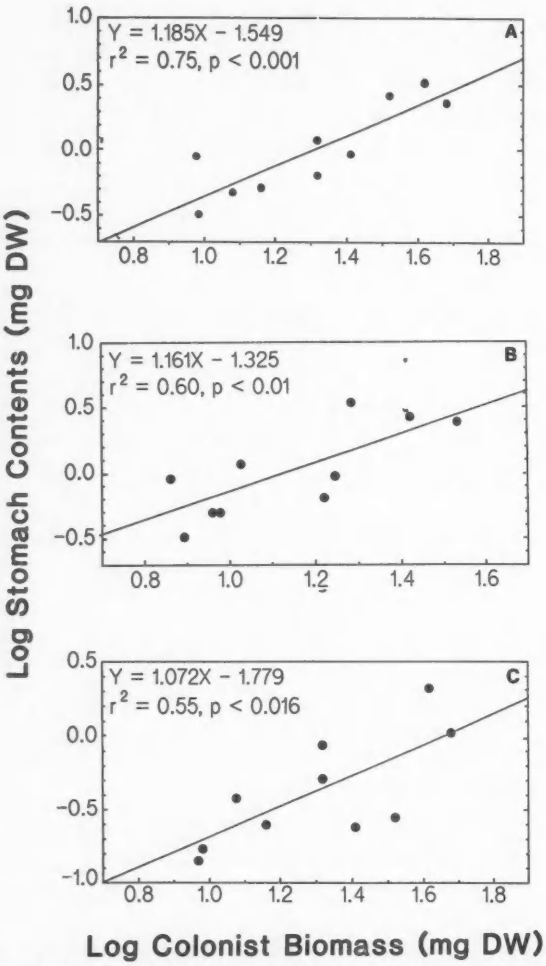

FIG. 2. Relationships between bluegill stomach contents (mean biomass) and various measures of macroinvertebrate colonization of artificial macrophytes. (A) Mean total biomass of colonists. (B) Mean biomass of most active taxa. (C) Mean biomass of invertebrates <0.5 mg DW body mass. Equation of the line, coefficient of determination, and significance level for linear regression analysis are given for each relationship.

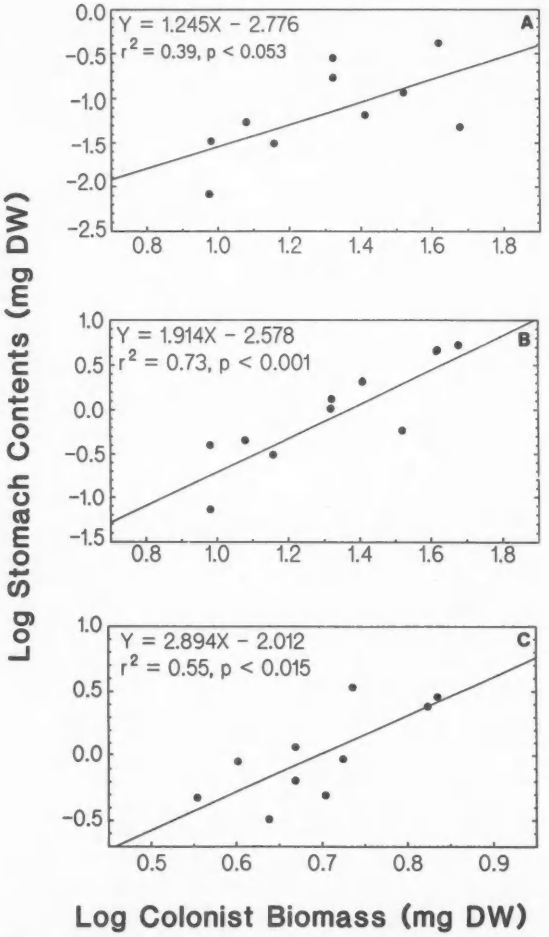

FIG. 3. Relationships between various measures of bluegill stomach contents and mean total biomass of macroinvertebrates colonizing artificial macrophytes. The representation of stomach contents, in each case, has been modified to reduce among-fish variation in foraging (see Methods). (A) Biomass of stomach contents/gram fish biomass. (B) Stomach contents (mean biomass) for age II fish (55–75 mm FL). (C) Stomach contents (mean biomass) for age III fish (76–102 mm FL). Equation of the line, coefficient of determination, and significance level for linear regression analysis are given for each relationship.

There have been a few previous attempts to predict food intake and related parameters from measures of food abundance, with which our results can be compared. Mittelbach (1988) found that final prey density in experimental predation cages explained 43% of the variation in mean sunfish growth (bluegills and pumpkinseeds, *Lepomis gibbosus*, combined), but the explained variance was associated largely with a single data point. Mittelbach (1981a) found that 71% of the variation in the mass of bluegill stomach contents could be explained by energetic returns predicted from an optimal foraging model that was applied to untransformed conventional measurements of littoral prey density. In later studies using optimal foraging models, Mittelbach (1983, 1988) found that model-modified conventional benthic data were also able to explain much of the variation in bluegill growth (r^2 = 0.88 and 0.57, respectively). In comparison with these results, a regression using our total colonist biomass data, untransformed to make it comparable with Mittelback's untransformed benthic data, explained 77% of the variance in total stomach contents. This high coefficient of determination suggests that artificial substrate colonization provides estimates of prey availability that are as good as conventional measures modified by optimal foraging models. It is also plausible that substituting artificial substrate colonization data for conventional benthos density estimates in Mittelbach's optimal foraging models could even further increase the precision of model predictions.

Since artificial substrate measures of available benthos are convenient and explain bluegill food intake as well or better than traditional, more awkward and costly methods, a more

thorough evaluation of the general utility of the artificial substrate method would be worthwhile. Our ability to detect within-lake variation in macroinvertebrate colonization suggests the potential for detecting between-lake differences in prey availability, so that colonist biomass might serve as a general index of fish food availability across many lakes. Artificial substrates could also be calibrated to predict stomach contents or growth (e.g. from daily growth annuli using otoliths) for other widespread species such as pumpkinseed or yellow perch (*Perca flavescens*), or for an entire fish assemblage.

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Variability, Spatial Patterns and Scales of Similarity in Size–Frequency Distributions of the Northern Shrimp (*Pandalus borealis*) and its Migrations in the Gulf of St. Lawrence

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The causes of high variability of length–frequency distributions (LFDs) of the northern shrimp in the Gulf of St. Lawerence were investigated with spatial statistics. The LFD is considered as a multivariate whose spatial structure was analyzed with cluster analysis, dissimilarity variograms and multivariate correlograms. Data were collected in 1984, 1985, and 1987 on the three main shrimp grounds: Sept-Iles, North-Anticosti, and Esquiman. The LFDs were spatially autocorrelated over a wide range of scales, and well-defined homogeneous assemblages were observed in each region every year. The autocorrelation signature revealed that the spatial organization resulted from large gradients, partially related to the topography of the shrimp grounds, which probably resulted from the ontogenic depth-migration of the shrimps. The specific mean length of cohorts consistently increased along the spatial gradients, up to about one-half of the annual growth increment. This was attributed to the fact that the migration routes passed through an increasing temperature gradient, which, directly or indirectly, accelerated their growth. Significant regional and interannual differences in the relative proportion of the modal components of LFDs were noted, and were ascribed to fluctuations in the success and distribution of recruitment and survivorship.

Les causes de la forte variabilité des distributions de fréquence de longueur (DFLs) chez la crevette nordique du Golfe du Saint-Laurent sont étudiées à l'aide de statistique spatiales. Les DFLs sont considérées comme une multivariable dont on étudie la structure spatiale au moyen de groupements hiérarchiques, de variogrammes de dissimilarité et de corrélogrammes multivariables. Les données ont été récoltées en 1984, 1985, et 1987 dans les trois principales régions de pêche: Sept-Iles, Nord-Anticosti, et Esquiman. Les DFLs étaient toujours autocorrélées suivant un large continuum d'échelles spatiales, et des assemblages homogènes bien définis ont été observées chaque année dans chacune des trois régions. La forme d'autocorrelation indiquait que l'organisation spatiale provenait de large gradients, modulés par la topographie des régions. Ces gradients résultaient vraisemblablement de la migration ontogénique des crevettes vers les fonds plus profonds. Les longueur moyennes spécifiques des cohortes ont montré un accroissement systématique le long des gradients spatiaux, représentant jusqu'à la moitié de la croissance annuelle. Ceci fut attribué au fait que les routes migratoires croisaient un gradient positif de température, qui accélérait, directement ou indirectement, leur croissance. Des différences régionales et interannnuelles considérables de la proportion relative des modes composant les DFLs ont été notées, et furent attribuées à des fluctuations dans le succès et la répartition du recrutement et de la survie.

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For the management of the fishery of the northern shrimp (*Pandalus borealis*) population in the Gulf of St. Lawrence (Fig. 1), the biomass is periodically evaluated by means of a direct sampling program, using a random stratified plan (Savard 1989). The mass of shrimp per stratum is easily estimated according to the swept area method (see Mackett 1973) but the number of individuals by length–class and age–group are much more difficult to evaluate accurately. The complication is due to the high variability of length–frequency distributions (LFDs), from which cohort strengths are derived, by the extraction of modal components (Macdonald and Pitcher 1979). This variability is not particular to the Gulf of St. Lawrence but common to most northern shrimp populations (e.g. Simard et al. 1975; Horsted and Smidt 1956; Apollonio et al. 1986).

For population dynamics study, precise estimates of lengths and numbers at ages are needed. In the present case, these estimates significantly change from sample to sample, which considerably limits the possibility of using LFDs to derive the age structure of the population. Typically, they vary in time (Fig. 2A), in space, at large (Fig. 2B) and small (Fig. 2C) scales, and with depth (Fig. 2A–D). Attempts to reduce variability by summing up LFDs on a regional basis or on depth intervals have been disappointing. The polymodal shape of the resulting LFD was always smoothed (e.g. Fig. 2D), and the mean length and relative proportion of cohorts extracted changed unpredictably with the arbitrary chosen integration unit (depth interval, strata, etc.). This stressed the need of an integrated study of the variability structure, aiming to identify its principal components and their deriving mechanisms, which can be taken into account while sampling and analyzing the LFDs for population dynamics modeling. This is the main objective of the present paper.

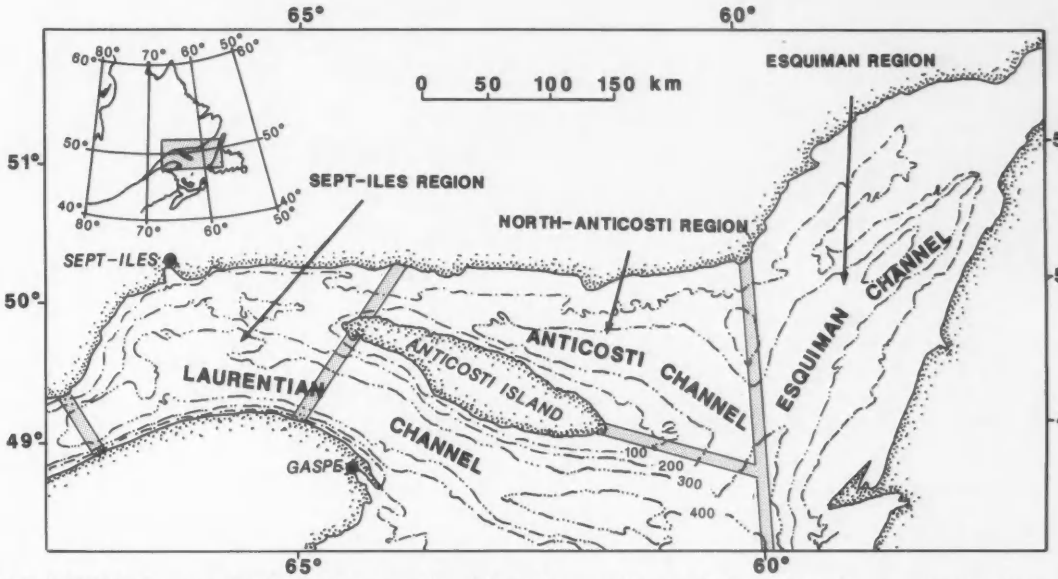

FIG. 1. Gulf of St. Lawrence showing the three channels and the regions sampled. Boundaries divide the five management units to which a stratified random subsampling design was applied. Depth contours in metres.

We hypothesize that LFD variability results from three main sources: (1) the precision of the sampling and measuring techniques, (2) spatial patterns, and (3) temporal fluctuations. The efficiency of trawl sampling is clearly far from ideal. Two trawl samples from the same point are not expected to give exactly the same LFD, because of the low performance of the sampler and unresolved small-scale patchiness. Subsampling the catch and measuring shrimps are two subsequent steps likely increasing the error. The sum of all these errors represents the residual variability. Another source of variability superimposed is due to the spatial structure present on the sampled area. If the LFDs are not randomly distributed, as generally assumed, but exhibit some type of spatial organization, they will be patterned according to the type of structure involved: patches, gradients, etc. Various physical/biological processes such as migrations, competition, predation, fishing pressure, success and distribution of recruitment act together to generate spatial structures, and nonrandom distribution of size-classes is the general rule. Finally, the last evident source of LFD variability are the annual fluctuations of age-group strengths, due to the variations in their recruitment and survivorship, which can also exhibit a spatial structure.

To analyze LFD variability in the northern shrimp population in the Gulf of St. Lawrence, the LFD is considered as a multivariate whose structure is examined with cluster analysis and multivariate spatial statistics. Spatial patterns are identified by their autocorrelation signatures (see Margalef 1979; Sokal 1986), from which inferences on the generating processes are derived. Specific objectives of the analyses were: (1) to test for the presence and persistence of spatial patterns and to describe the patterns if any, (2) to measure the residual level of variability and the scales of organization in space and along depth, (3) to extract the ecological information on the local population evidenced by the LFD structure and (4) to suggest strategies to take the structure into account when sampling and analyzing LFDs for population dynamics modeling.

Methods

Study Area and Shrimp Population

The Gulf of St. Lawrence bathymetry is characterized by the ~400 m deep Laurentian Channel which divides into three arms in the northern Gulf: the Esquiman Channel, the Anticosti Channel, and the remainder of the Laurentian Channel, which widens in the Sept-Iles region in the northwestern Gulf (Fig. 1). Northern shrimps are abundant between 200 and 300 m in these three depressions, especially at their upstream end, where fishing is concentrated. Water temperature has a typical three-layer structure: (1) an upper layer (~50 m) where surface temperature fluctuates from freezing point in winter to a maximum of about 15°C in summer; (2) a cold intermediate layer (~50–100 m) where minimum temperature can reach −2°C; and (3) a warmer layer (~100–500 m) where temperature increases with depth from ~1°C to a maximum of ~6°C. This latter gradient determines the bottom temperature on the shrimp grounds.

Hatching occurs during a short 2–3 wk period from mid-April to mid-May, during the ice break up and the spring phytoplankton bloom. Benthic settlement of pelagic larvae occurs at the end of the summer. The hermaphroditic life cycle has an initial male phase lasting 4 yr. Sex inversion happens mostly during winter, gonads develop during summer and extrusion of eggs and fecundation occur at the end of the summer. A proportion of the ovigerous females migrate to shallow areas (150–200 m), where they form patchy aggregations from December to April, notably in the Sept-Iles region. Fishing usually takes place from spring to mid-fall and landings reached 14 000 t in 1988 (Savard 1989).

Sampling and Laboratory Measurements

The data analyzed come from trawl surveys carried out during early fall of 1984, 1985, and 1987 for biomass estimation.

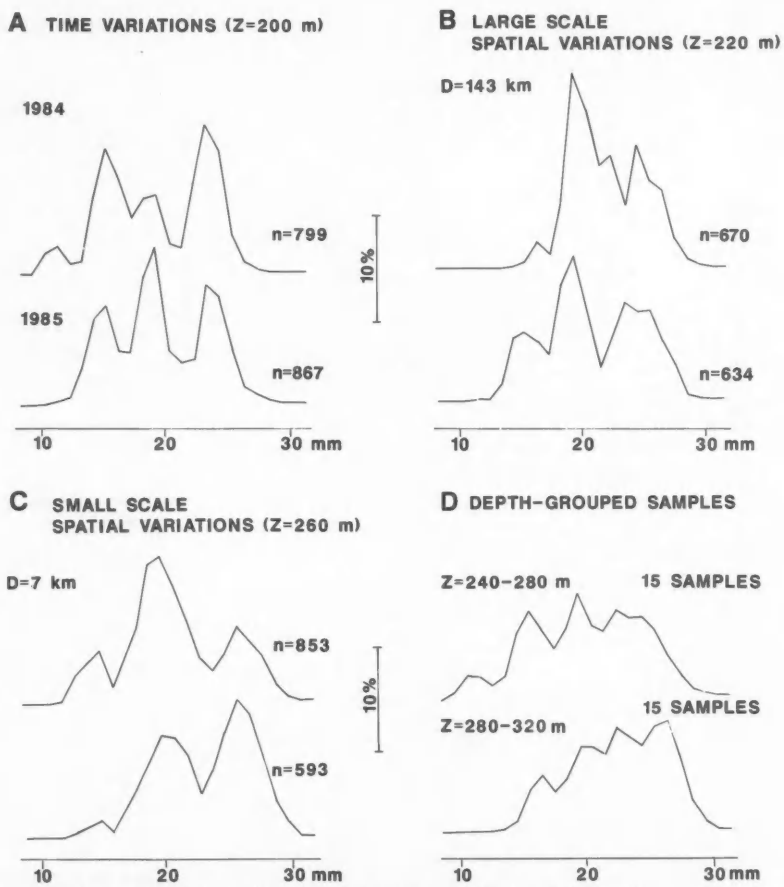

FIG. 2. Typical variations of samples of length–frequency distributions (CL in millimetres, frequency in percent) of northern shrimp in the Gulf of St. Lawrence in time (A), space (B) and small scales (C), and of depth-grouped samples (D). Same sampling gear and techniques, same shrimp ground and period of the year. N = No. of shrimps measured per sample, Z = depth in metres.

Stations were chosen according to a stratified random sampling design applied to five management units, the three most important being the Sept-Iles, North-Anticosti, and Esquiman regions (Fig. 1). In 1987, the North-Anticosti region was not sampled. The number of stations sampled were 124, 126, and 96 for the 3 yr, respectively. The strata were set from the 1970s spatial patterns of catch rates, combined with depth and bottom sediment formation (Fréchette and Labonté 1981). For spatial analysis, this sampling scheme has the drawbacks of presenting clumped distributions of samples, varying each year, instead of a constant grid, and is limited to trawlable grounds only.

Sampling was done with a Western-2A bottom trawl towed by a 27-m shrimp trawler. The vertical and horizontal maximum openings of this trawl are 3.5 m and 13.5–15 m, respectively. In 1987 the openings were monitored with a Scanmar Model 4002 acoustic net-sonde system. The towing speed was about 3.5 knots for an average of 30 min, which gives a sampling surface of about 0.05 km^2 (3500 × 14 m). Stretched mesh aperture was 38 mm and the cod-end was covered with an additional 19-mm mesh net. The 38-mm mesh retains 100% of the shrimps with a carapace length greater than 23 mm and the cover doubles the retention of the individuals longer than 18.5 mm CL (Labonté and Fréchette 1978). Smaller individuals are subject to the sigmoid net retention function. Sampling was done only during daytime because of vertical migrations of the shrimps off the bottom at night (Barr 1970; Apollonio et al. 1986).

On board, the catch was sorted, weighed, and a random subsample of 5 kg (~500 individuals) of shrimp was taken. Their sex and developmental stage were determined and their carapace length (from the posterior margin of eyestalk to the posterior mid dorsal edge) was measured to the nearest 0.1 mm with an electronic vernier caliper linked to a microcomputer. The resulting frequency distribution of length-classes of 0.1 mm was filtered with a 0.5-mm moving average to smooth out the measuring error. Then the frequencies were grouped in classes of 1.0 mm in order to reduce the data for subsequent numerical analyses, which gave 24 classes, from 8 to 32 mm.

Description of Spatial Patterns: Cluster Analysis

The next step was to sort the samples according to their similarity in LFD, with nonparametric methods. Considering the

LFD as a multivariate with 24 dimensions (f_i; $i = 1$–24) given by the length-classes, a dissimilarity index between the samples (x_j; $j = 1-n$) was first computed. The Bray and Curtis (1957) index (d) was used. The dissimilarity between samples x_1 and x_2 is:

$$d(x_1,x_2) = \frac{\sum_{i=1}^{24} f_{i1} - f_{i2}}{\sum_{i=1}^{24} (f_{i1} + f_{i2})}$$

where f_i is the relative frequency (percent) of the corresponding length-class. This dissimilarity index ranges from 0, when the two samples have the same LFD, to 1.0, when they are totally different. It has the property of excluding the double zeros from the comparison. The symmetric matrix ($D_{n\times n}$) obtained gives the dissimilarity between all pairs of combinations of the n samples.

The complete linkage (furthest neighbor) algorithm was chosen to sort the samples according to their similarity, because it maximizes the within group homogeneity and the between group discontinuity. To join a group, a sample (or a group) must present the required similarity level with all members of the group (for details on cluster analysis, see Legendre and Legendre 1983). Then, the resulting four to five clusters corresponding to the most important branches of the grouping tree were mapped to look at their spatial organization. The mean LFD of the groups was computed and their modal components were extracted by fitting a series of normal curves according to Macdonald and Pitcher (1979).

This analysis was done first for the total LFDs (males + females combined), and then for the LFDs of males and females separately. The chord length dissimilarity index and the flexible linkage algorithm with $\beta = -0.25$ (see Legendre and Legendre 1983) were also computed and gave final results very similar to those from the above method.

Analysis of Structures: Mantel Tests, Variograms, and Correlograms

To test for nonrandom spatial organization and to determine the type of spatial structure, the Bray and Curtis dissimilarity matrices were submitted to further analyses. Mantel tests (Mantel 1967; Sokal 1979a) using the normalized Mantel statistic (r, Smouse et al. 1986) were computed to compare these matrices, $D_{n\times n}$, with various model matrices, $Y_{n\times n}$.

$$r = \left[\sum_i^n \sum_j^n (D_{ij} - \bar{D})(Y_{ij} - \bar{Y})\right] \Big/ \left[\sum_i^n \sum_j^n (D_{ij} - \bar{D})^2 \sum_i^n \sum_j^n (Y_{ij} - \bar{Y})^2\right]^{1/2}$$

for $i \neq j$; where i and j are the lines and columns of the matrices and $\bar{D}$ and $\bar{Y}$ are the means. Because the matrices are symmetric, r is computed with the $n(n-1)/2$ distinct entries only. This statistic is analog to a Pearson correlation coefficient calculated between the paired elements of the two matrices. It ranges from -1.0 to 1.0. Its probability was obtained from comparison to a Monte Carlo null distribution, computed from 250 random permutations of the rows and columns of one matrix.

In order to test if the spatial organization of similarity in LFDs could be the result of chance alone (i.e. no significant spatial autocorrelation), the first model matrices used were the geographic distances between the stations for each region. Then, to examine the type of spatial structure responsible for the autocorrelation (mosaic of patches, gradients, etc., see Sokal and Oden 1978; Sokal 1979b, 1986) and to measure the degree of LFD similarity and autocorrelation at various scales, dissimilarity variograms (Mackas 1984) and Mantel correlograms (Sokal 1986; Oden and Sokal 1986) were computed. Spatial dissimilarity variograms simply expressed the variation of dissimilarity against the geographic distances between stations. No attempt was made to fit a function to the variograms. The Mantel spatial correlograms expressed the variations of r against the geographic distances between stations. These correlograms were computed from a series of binary matrices of classes of distances of 10 or 20 km. For each class of distances, the normalized Mantel statistic r was computed using a model matrix whose elements were 1 where the distance was corresponding to the class of distances considered, and 0 elsewhere (Sokal 1986; Oden and Sokal 1986). Because this latter binary index is the opposite of a distance, in order to have positive r for positive autocorrelation, the similarity matrices ($S = 1 - D$) were used instead of the dissimilarity matrices D.

Similar sequences of Mantel's tests, variograms and correlograms were used to study the similarity in LFDs using depth separation (classes of 10 m) between stations.

Partial Mantel tests (analog to partial correlation, see Smouse et al. 1986; Legendre and Troussellier 1988) were also computed to see if the spatial autocorrelations were still significant when controlled for depth.

Results

Spatial Patterns of LFDs and Time Variations

Every year, five different groups (a to e) of total LFDs (males + females) were separated by the cluster analysis at dissimilarity levels of 0.5 to 0.8 (Fig. 3). Their mean LFDs, sorted in an increasing order of modal mean lengths, are presented in Fig. 3. Every year, four overlapping modes could be extracted from the mean LFD of the first cluster (group a), generally with decreasing amplitude with increasing length. In the second group (b), the four modes were still present, except in 1984, but their proportions changed markedly, with the dominance often shifted to the third mode. The third and fourth groups (c and d) followed the same trend towards larger shrimps, resulting in the dominance of the fourth mode. In the fifth group (e), the first mode vanished and more than 75% of the individuals belonged to the third and fourth modes. The gradual disappearance of the young individuals from group a to e was observed every year. In contrast, the relative proportion of the modes in each group exhibited important interannual variations.

Four modal components were extracted from the LFDs of the groups (Table 1). Three modes of males (M1–M3) were identified, having mean lengths of 11–13, 16–18, and 19–21 mm. Since the females could not always be separated into primiparous and multiparous individuals, because most of them had extruded eggs, only one modal component (F) with a mean length of 23–26 mm was estimated, even though it represented at least two year-groups. The first and last modal components of LFDs were difficult to separate when their proportions were small. An indication of nonuniform growth rate was revealed by a significant (2–3 mm) and consistent increase of the mean

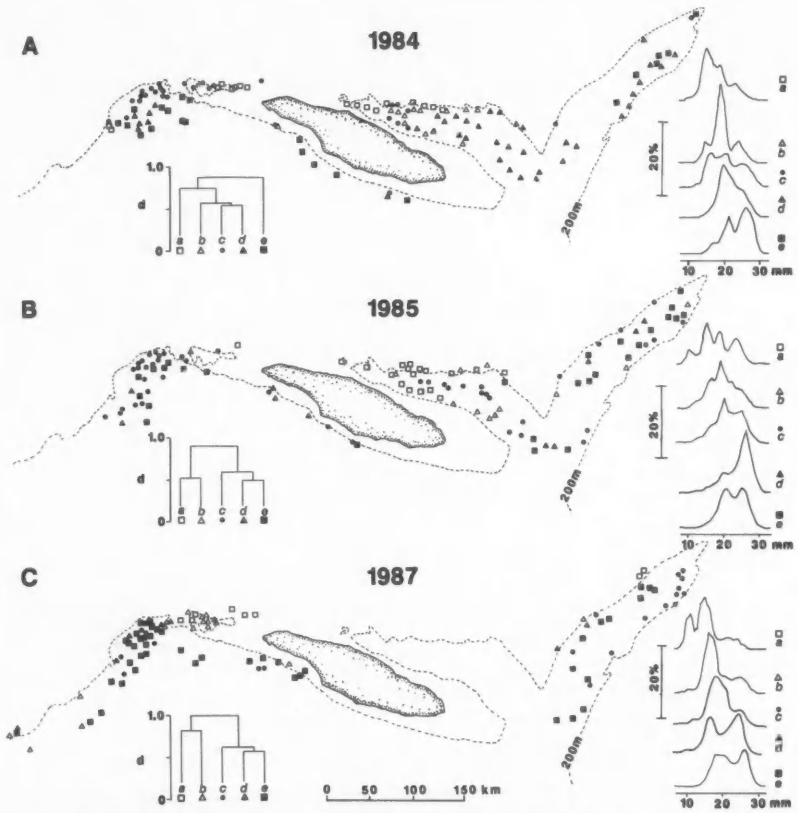

FIG. 3. Results of the cluster analysis showing the spatial organization of similarily of the LFDs of the total shrimps in early fall of 1984, 1985, and 1987 in the Gulf of St. Lawrence. Mean LFDs of the five most important branches (a to e) of the dendograms. Carapace length in millimetres, frequency in percent.

length of every modal component from LFD group *a* to group *e* (Table 1).

Every year the stations belonging to a given LFD group were not randomly distributed but clearly clumped, with the closest samples tending to belong to the same group (Fig. 3), which clearly indicated that the LFD were spatially organized. In general, the distribution of the groups reflected the regional bathymetry; the LFDs with the highest proportions of small individuals and with the smallest modal mean lengths were found in the shallower depths at the channel borders, and conversely for the deeper parts. Sometimes, one group was present in one region only (e.g. Fig. 3A, group *b* Fig. 4A, groups *a*,*b*), which indicates some regional exclusivity in LFDs patterns.

The spatial distribution of the LFD assemblages for males and females separately (Figs. 4 and 5) corroborates the above results. The females and males presenting the largest mode-specific mean lengths were found in same locations, and conversely, which indicated similar local effects on all modal components. The increase of female modal lengths through the LFD groups does not result from a gradual shift from primiparous to multiparous spawners, since both stages were found in all groups when their separation (from the presence of sternal spines on the shrimps, (McCrary 1971)) was possible (1987 only).

Tests of Autocorrelations

Mantel tests confirmed the tendency of LFDs to be spatially autocorrelated every year in Sept-Iles and North-Anticosti, but not in Esquiman (Table 2, column Geo.). Since the diffusion of similarity was not isotropic but clearly organized along bathymetric contours (Figs. 3–5), it seemed appropriate to test the autocorrelation of LFDs using depth separations between stations instead of geographic distances. These latter tests (Table 2, column D) showed significant autocorrelation in all three regions and for the whole Gulf, every year, except for 1984 in Esquiman where r is significant at $p<0.05$. The depth structure of LFD was therefore very strong. The spatial autocorrelations noted above could thus simply reflect the distribution of the stations along the bathymetry of the basin; i.e. close LFDs are spatially autocorrelated because they are at similar depths. The significant spatial autocorrelations of the station depths observed in North-Anticosti and Sept-Iles (Table 2, symbol *) would support this hypothesis. To test it, partial Mantel tests were computed and showed that spatial autocorrelations in both regions were still significant when controlled

TABLE 1. Parameters of the modal components of the mean LFDs of the groups of samples classified with the cluster analysis (Fig. 3). Mean (M) carapace length; 95% range (R) of the normal distribution, percent (P) of total numbers according to Macdonald and Pitcher (1979). M1–M3 = males; F = females.

Groups		Modes			
		M1	M2	M3	F
1984-a	M	11.1	15.7	19.2	23.3
	95% R	9.3–12.9	13.2–18.2	17.0–21.4	18.8–27.8
	P	0.02	0.50	0.24	0.24
1984-b	M		16.0	19.7	24.6
	95% R		13.8–18.2	17.7–21.7	21.1–28.1
	P		0.16	0.59	0.25
1984-c	M		16.7	20.6	25.9
	95% R		13.8–19.6	17.3–23.9	22.0–29.8
	P		0.29	0.37	0.34
1984-d	M		16.2	20.5	25.0
	95% R		14.0–18.4	17.4–23.6	21.1–28.9
	P		0.03	0.56	0.41
1984-e	M		17.6	21.5	26.5
	95% R		14.9–20.3	19.0–24.0	23.2–29.8
	P		0.10	0.31	0.59
1985-a	M	11.0	15.5	19.1	23.7
	95% R	9.0–13.0	13.1–17.9	17.3–20.9	20.2–27.2
	P	0.12	0.35	0.22	0.31
1985-b	M	13.2	16.0	19.4	24.1
	95% R	10.5–15.9	14.2–17.8	16.3–22.5	20.0–28.2
	P	0.08	0.16	0.46	0.30
1985-c	M	13.4	16.7	20.2	24.7
	95% R	11.0–15.8	14.5–18.9	17.7–22.7	20.8–28.6
	P	0.05	0.14	0.35	0.46
1985-d	M		17.2	21.3	26.5
	95% R		12.7–21.7	17.6–25.0	23.4–29.6
	P		0.08	0.24	0.68
1985-e	M		16.1	20.6	25.4
	95% R		13.2–19.0	17.3–23.9	21.7–29.1
	P		0.04	0.42	0.54
1987-a	M	11.1	15.6	20.8	24.3
	95% R	9.3–12.9	12.7–18.5	16.9–24.7	21.0–27.6
	P	0.24	0.56	0.12	0.08
1987-b	M	11.5	16.8	21.0	25.3
	95% R	9.3–13.7	14.1–19.5	17.9–24.1	21.8–28.8
	P	0.03	0.60	0.17	0.20
1987-c	M	13.3	18.4	21.3	25.6
	95% R	10.9–15.7	15.5–21.3	18.9–23.7	22.5–28.7
	P	0.05	0.51	0.26	0.18
1987-d	M	11.4	17.5	21.0	24.9
	95% R	9.4–13.4	14.4–20.6	18.3–23.7	21.6–28.2
	P	0.02	0.39	0.14	0.45
1987-e	M		18.2	21.3	26.3
	95% R		14.9–21.5	18.0–24.6	23.4–29.2
	P		0.26	0.32	0.42

for depth (Table 2, column Geo.; values in square brackets). Bathymetry alone could therefore not explain all the organization of LFDs in Sept-Iles and North-Anticosti. An additional spatial component was also present.

Variations of LFD Similarity with Distance and Depth

The initial level (intercept) on a dissimilarity variogram defines the unresolved residual variability, d_e, which represents the expected minimum dissimilarity between replicate samples (zero distance). This level was the same, $d_e = \sim 0.18$, for both Sept-Iles and North-Anticosti and over the 3 yr of sampling (Fig. 6 A,C). For Esquiman too few stations were close enough to estimate the initial value on the variograms (Fig. 6E). The background variability in LFD seemed therefore homogeneous in the Gulf and was stable over the years, which means that the efficiency of the sampling technique and its resolution of the small scale patchiness were stable.

The range of autocorrelation (Ra, Fig. 6A) is given by the scale at which the variogram levels off. In Sept-Iles and North-Anticosti the variograms did not level off (Fig. 6A, C) and the dissimilarity in LFDs kept increasing up to the upper limit of our observation window, meaning that the LFDs influenced each other over the entire region sampled. The maximum dissimilarity reached was about the same, $d_m = \sim 0.45$, but it was reached at a larger range in North-Anticosti (~170 km, Fig. 6C) than in Sept-Iles (~120 km, Fig. 5C). The variogram slope was therefore higher in Sept-Iles, indicating that for a similar geographic separation, the LFDs there would differ more than in North-Anticosti. The nonmonotonic variograms in Sept-Iles in 1984 and 1985 are indicative of a lack of continuity in the spatial decrease of LFD similarity.

In Esquiman, the variograms leveled off at a dissimilarity of ~0.3–0.4 corresponding to a range of ~50 km (Fig. 6E). The scale of the local structure was therefore smaller there than in the other two regions; the influence of LFDs on each other being limited to about 50 km.

The Mantel correlograms (Fig. 6B,D,F) are instructive about the possible structure responsible for the autocorrelation. Positive autocorrelation at small scales combined with negative autocorrelation at large scales reflect a large gradient, i.e. the spatial structure or the patch size is larger than the observation window. Such large gradients seemed to be the only spatial organization present in LFDs of North-Anticosti and Sept-Iles. In Esquiman, significant autocorrelation at small scale and random variations of r at large scales (Fig. 6F) indicated small patches of LFDs. The radius of these patches is generally estimated from the scale at the first zero crossing of the autocorrelation function, which was about 25–35 km (Fig. 6F).

When the LFD similarity is considered in depth intervals instead of geographic distances (Fig. 7), the variograms and the correlograms were similar in all regions, which reflects that the LFDs were similarly organized along depth. The background variability of similarity ($d_e = \sim 0.20$–0.23, Fig. 7) computed this way was slightly higher than in space, because of the variability added by the integrated mesoscale (10–100 km) spatial structures (Figs. 3–6). Sept-Iles had somewhat lower and more variable diffusion of similarity (~ 1.1–$2.2 \times 10^{-3} m^{-1}$) than the two other regions ($\sim 2.5 \times 10^{-3} m^{-1}$), however. The LFDs influenced each other over the whole depth range sampled in each region (70 to 110 m).

Discussion

As postulated, the LFD variability of the northern shrimp in the Gulf of St. Lawrence can be sorted out in three components: a background level of variability, spatial structures, and temporal variations. The background level of variability was stable in time and space and represented a dissimilarity level of about 0.18. This result was reassuring because it indicated a constancy in the sources of residual errors. Therefore, the LFD variability cannot be ascribed only to the low and/or variable performance of the sampling techniques or to unresolved small-

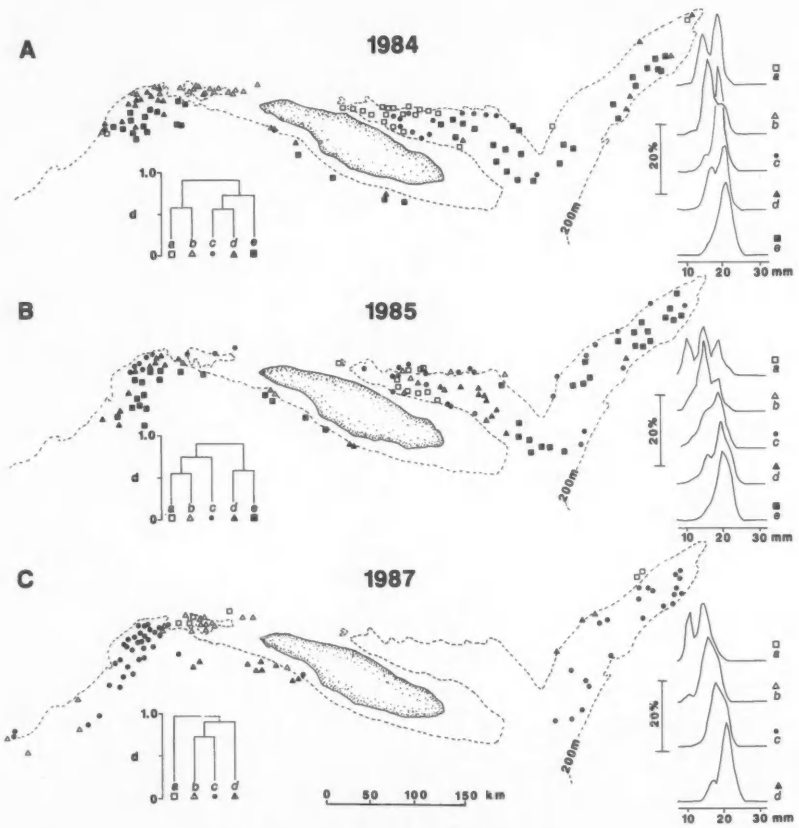

FIG. 4. As Fig. 3 but for male shrimps only.

scale patchiness. Every year and in every region, they produced the same error level, even though the total biomass varied substantially (Savard 1989). Above this small-scale background variability, LFDs were never randomly organized. Spatial structure was always present, and was responsible for an increase of LFD dissimilarity up to 0.40–0.45 in each region. The spatial structures involved were persistent monotonous gradients, largely (but not exclusively) related to the particular bathymetric shape of the regional basins sampled, as shown by the autocorrelation structure with depth (Fig. 7). The differences in variogram slopes between regions are also thought to be partly related to the particular shape of the basins. The range of influence of LFDs on each other extended to the whole depth range (70–110 m) sampled in each region. Except for Esquiman, the structure was also stretched over the whole region, corresponding to scales of 120–170 km. Finally the analysis also showed important general changes of LFD from year to year.

Ecological Inferences from the Spatial Structures

The spatial structures and the autocorrelation characteristics found can be used to infer on the processes driving the variability of LFDs. The most consistent feature observed was the organization of similarity in LFDs along the depth, as revealed by the Mantel tests (Table 2) and the variograms (Fig. 7). This persistent organization cannot be attributed to physical processes which change annually or regionally, such as circulation patterns. This depth arrangement is more likely influenced by the biological characteristics of the northern shrimp, and the process involved is most probably its ontogenic horizontal "migration" to deeper grounds. Juveniles are generally found at different depths and locations than older individuals (see Shumway et al. 1985 and references therein).

Assuming that this ontogenic migration is the main source of the consistent gradients of LFD dissimilarity observed along depth, the scales at which the maximum dissimilarity (d_m) was found can be considered as an indication of the ranges off the migration in the surveyed areas. These scales vary from 70 to 110 m, as shown by the ranges of autocorrelation (Ra) of LFDs (Table 3). The time needed to develop the maximum dissimilarity of LFDs can be estimated from the number of age-classes mainly involved in the migration (Figs. 3 and 4). The maximum dissimilarity was found between the LFD-group *a* (Fig. 3 and 4), composed of three male components (M1–M3, Table 1) and one female component (F), and the LFD-group *e*, where modes M1 and M2 were absent (Figs. 3 and 4). Two years therefore separate the start and the end of the migration to the deeper grounds. By dividing the ranges of migration by this time period, we conclude that minimum migration rates to deeper waters were 55 $m \cdot yr^{-1}$ in Sept-Iles, 45 $m \cdot yr^{-1}$ in North-Anticosti and 35 $m \cdot yr^{-1}$ in Esquiman.

The migration had also an additional spatial component and the horizontal migration rates can be obtained as above, by

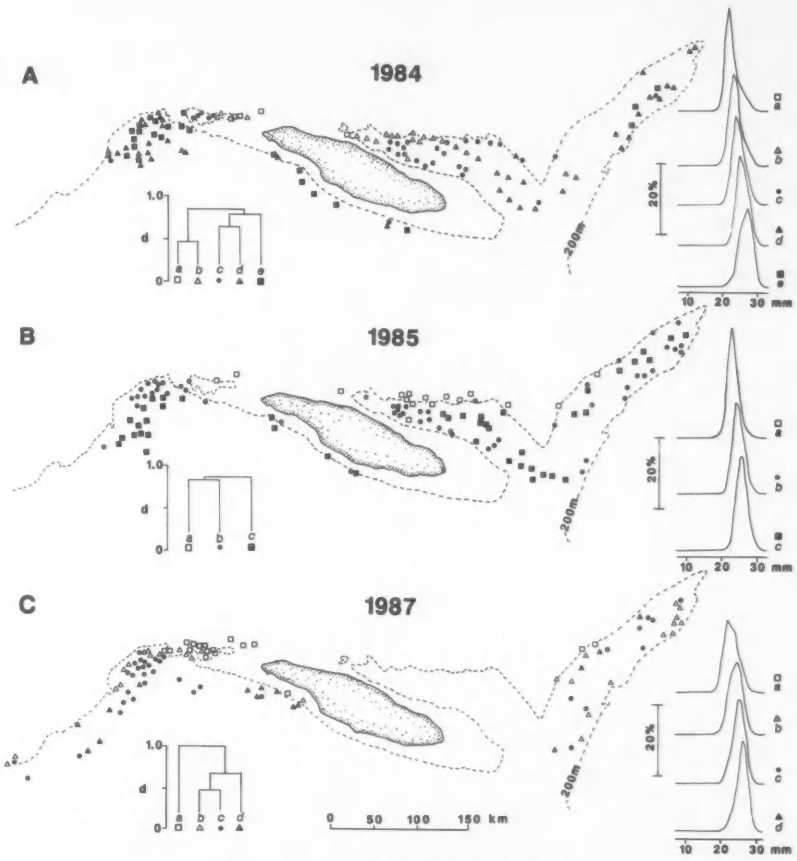

FIG. 5. As Fig. 3 but for female shrimps only.

TABLE 2. Normalized Mantel statistic (r) between the dissimilarity matrices and the model matrices of geographic separation between the stations (Geo.) and depth (D.) differences. Underlined values are significant ($p<0.01$). * indicates significant ($P<0.01$) spatial autocorrelations of the station depths. Square brackets identify partial spatial r controlled for depth.

	Model matrix						
	Sept-Iles		North Anticosti		Esquiman		Gulf
Year	Geo.	D.	Geo.	D.	Geo.	D.	D.
1984	0.41 [0.32]	* 0.36	0.51 [0.50]	* 0.49	−0.02 [−0.02]	* 0.25	0.25
1985	0.21 [0.25]	* 0.27	0.46 [0.46]	* 0.43	0.00 [0.04]	0.47	0.32
1987	0.46 [0.25]	* 0.59	—	—	0.11 [0.16]	* 0.46	0.34

dividing Ra by 2 yr, which gives estimates 60, 85, and 25 km·yr^{-1}, for Sept-Iles, North-Anticosti and Esquiman, respectively.

The migration routes can be traced from the decrease in the percentage of small individuals and the increase in proportion of large individuals in LFDs. From the spatial patterns shown in Figs. 3 and 4, the shrimps appear to move southwest–south in Sept-Iles, southeast in North-Anticosti and across the channel in Esquiman. The source of recruits was not obvious for Esquiman, but important sources for the other regions appear to be the shallow grounds on each side of the western end of Anticosti Island. Whether these two regions share a common source is unknown, but, if it is the case, the LFD spatial organization (e.g. Figs. 3a; FLD*b*, 4A; LFD*a*,*b*) provides evidence of substantial differences in the allocation of recruits and/or their survival in each region. Moreover, important year to year variations in the success of the different cohorts are apparent from the LFD patterns (Figs. 3 and 4). This is evidence for important interannual fluctuations in recruitment and/or survivorship.

The consistent increase of the mean length of the female and older male modes along the inshore–offshore gradient (Table 1) is indicative of an additional effect of the migration. This apparent enhanced growth for the individuals following the migration route is substantial, about one-half of the annual increase in length. Whether this is an effect of better food conditions or warmer temperatures at deeper depths or both is beyond the scope of the present paper.

The traces of young male modes in the deep LFDs (Figs. 3 and 4) shows that some individuals migrate faster than the bulk of the population. In contrast, the presence of older shrimps

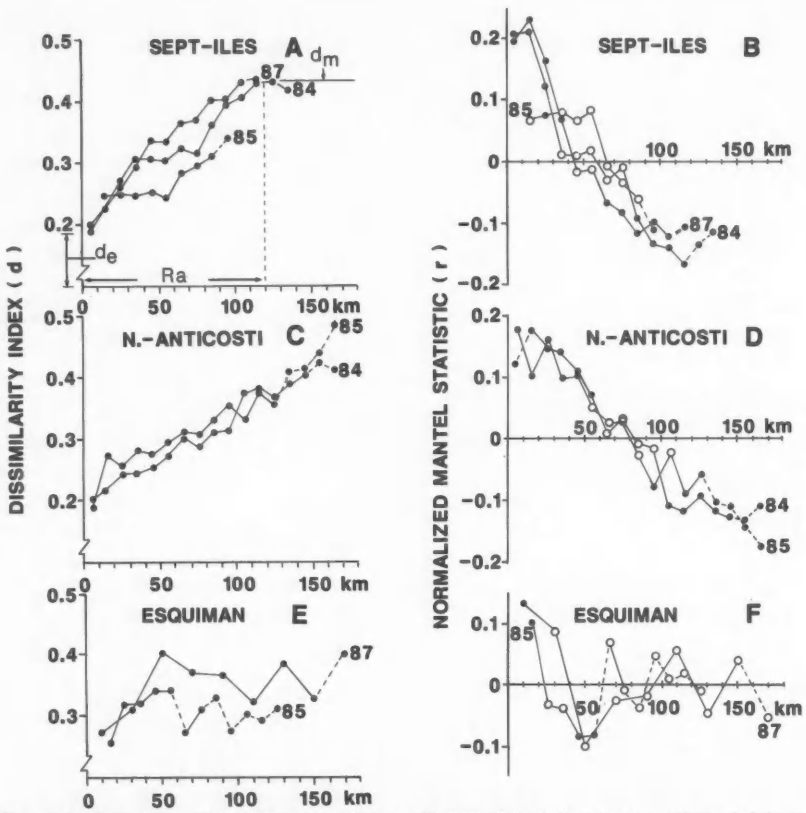

FIG. 6. Spatial variograms (A,C,E) and correlograms (B,D,F) of similarity of LFDs of the total shrimps for the three regions in the Gulf of St. Lawrence. Dotted lines indicate low n ($20<n<30$); open circles in correlograms are not significant ($p>0.01$). Ra = range of influence of autocorrelation; d_m = maximum dissimilarity observed, d_e = residual variability of dissimilarity.

(M3, F) in shallow LFDs (Figs. 3–5) demonstrates that some individuals do not seem to participate to the depth migration. Whether this sedentary behaviour is active or forced by some indirect process is unknown. From the distribution of LFDs (Figs. 3 and 4) it seems that more shrimps do not migrate from the head of Anticosti channel. There the deep flow is forced against the bathymetry (D. Lefaivre, Institut Maurice-Lamontagne, pers. comm.), which might help to retain some individuals upstream.

The present results do not fit perfectly to the migration model illustrated by an inshore development of immature males, which mature offshore and invert sex, before yearly migrating inshore during the egg-bearing period (Shumway et al. 1985). The above observations demonstrate that the model must include a substantial part of variability due to individuals in the populations. A more adequate representation of the migration in the Gulf of St. Lawrence is as follows: a gradual increase of the median depth of overlapping cohorts, where the shrimps are continuously separated according to their individual behaviour, along a route through a temperature gradient, which, directly or indirectly, enhances their growth.

Variability and Sampling Strategies

For controlling the variability when sampling and analyzing LFDs, the spatial structures must be taken into account. Because of the regional differences of LFDs and of the particular topography of the basins, the regions should be considered separately. Since the structures are continua, not discrete patches, and since important interannual fluctuations of the boundaries between LFD assemblages are common (Figs. 3–5), the division of regions in depth intervals or strata is not very useful. To adequately take the structure into account, maps of size classes should be constructed by means of interpolation methods.

The information from the variograms could be used to define cost-effective spatial sampling and analysis strategies. Because the most consistent organization observed was along the depth axis, depth should be used as the basis of sampling. To take the structure observed into account, only a few sampling points are needed because it was a simple gradient. The sampling density could be chosen to achieve a constant dissimilarity between samples. The variograms (Fig. 7) give the appropriate depth separation of samples for any chosen level of dissimilarity. For example, to achieve a dissimilarity of $d\approx0.25$ between the LFDs, the depth spacing of samples should be ~20–30 m. Then to obtain the same dissimilarity between samples in the horizontal plan, the appropriate spacing could be read from the spatial variograms (Fig. 6), which gives 20–40 km, depending on the region. Since the range of dissimilarity (d_e to d_m) and

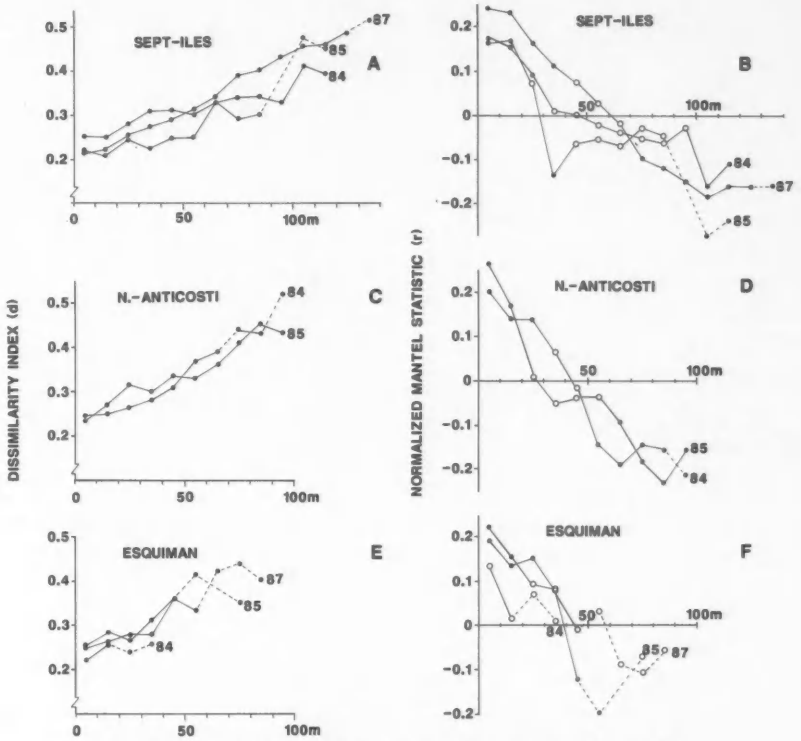

FIG. 7. As in Fig. 5, but with depth separation between samples as X-axis.

TABLE 3. Comparison of some parameters of the autocorrelation of LFD similarities in space and along depth in the Gulf. Approximate residual background variability at small scales (d_e) and maximum dissimilarity observed at large scales (d_m), range of influence of autocorrelation (Ra), and diffusion of similarity (variogram slope).

Parameter	Region		
	Sept-Iles	North Anticosti	Esquiman
d_e Space	0.18	0.18	<0.25
Depth	0.20–0.23	0.20–0.23	0.20–0.23
d_m Space	0.45	>0.42	0.34–0.40
Depth	0.45	0.45	0.42
Ra Space (km)	120	170	50
Depth (m)	110	90	70
Slope Space $\times 10^{-3}(km^{-1})$	1.5–2.4	1.6	2.4
Depth $\times 10^{-3}(m^{-1})$	1.1–2.2	2.5	2.5

variogram slopes were relatively stable, the sampling density chosen for the regions could be kept constant from year to year.

To estimate the size structure of the population, the same sampling plan could be used if the biomass is uniformly or randomly distributed or presents the same spatial organization as the LFD. If this is not the case and biomass varies more rapidly than the LFD, as often encountered, the above sampling plan could be used to sample LFD on the finer grid used for biomass estimation. Because LFD measurement requires more effort than catch weighing, such a strategy is advantageous.

Estimation of age-structure of the population is more complex, because the observed spatial variation of growth rate (Table 1) must be considered in the computation, besides the spatial differences in sizes and biomass. Further exploitation of LFD information must always carefully consider the spatio–temporal processes affecting the data. For example, because growth rate can be increased during migration, the growth of cohorts must be followed along the migration route. Samples from the same location through time would not be useful. Population dynamics modeling of northern shrimps in the Gulf of St. Lawerence thus clearly requires adequate consideration of these processes.

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Estimation of Submergent Plant Bed Biovolume using Acoustic Range Information

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Thomas, G. L., S. L. Thiesfeld, S. A. Bonar, R. N. Crittenden, and G. B. Pauley. 1990. Estimation of submergent plant bed biovolume using acoustic range information. Can. J. Fish. Aquat. Sci. 47: 805–812.

Acoustic measurements of the distance between the water surface, top of the aquatic plants, and bottom of the water column were made using chart recording echosounders. The vertical cross sectional area (m^2), height (m), biovolume (m^3), of aquatic plant beds and variances were computed for three surveys of Devils Lake, Oregon, in May, July, and September, 1986 when coefficients of variation for the plant bed biovolume estimates were 0.18, 0.05, and 0.06, respectively ($n = 14$). Coefficients of variation for plant biomass estimates (g/m^2) computed from SCUBA quadrat samples collected concurrently with the acoustic surveys, were 0.98 ($n = 48$), 0.81 ($n = 90$), and 1.05 ($n = 90$), respectively. The higher precision of the biovolume estimates allow for a 5- to 18-fold greater capability to detect a change in the mean. The lower costs of the biovolume estimates allow for a 10- to 33-fold greater precision-for-cost. The plant bed biovolume variable contains ecologically different information than the biomass variable in that it provides a direct estimate of the amount of aquatic habitat in a lake that is influenced by plants; it should prove useful for evaluating plant control practices and possibly for studying plant–fish interactions.

Des mesures acoustiques de la distance séparant la surface de l'eau et le sommet des plantes aquatiques, et le fond de la colonne d'eau ont été effectuées à l'aide d'échosondeurs graphiques. La superficie de la section verticale (m^2), la hauteur (m), le volume de végétaux (m^3) et les variations ont été calculés en mai, juillet et septembre 1986 dans le lac Devils en Orégon, lorsque les coefficients de variation du volume végétal étaient estimés respectivement à 0,18, 0,05 et 0,06 ($n = 14$). Les coefficients de variation des estimations de la biomasse végétale (g/m^2) calculés à partir des échantillons SCUBA de quadrats prélevés en même temps que les relevés acoustiques s'élevaient respectivement à 0,98 ($n = 48$), à 0,81 ($n = 90$) et à 1,05 ($n = 90$) pour les trois relevés. La précision plus élevée de l'estimation du volume végétal augmente de 5 à 18 fois la capacité de détection d'un changement dans la moyenne. Le coût inférieur de ces estimations permet d'obtenir un rapport coût-précision de 10 à 33 fois supérieur. La variable du biovolume végétal donne des informations écologiques différentes de celles de la variable de la biomasse en ce qu'elle donne une estimation quantitative directe de l'habitat aquatique d'un lac qui est influencé par la végétation; cela devrait se révéler utile pour l'évaluation des méthodes de contrôle des végétaux et, éventuellement pour l'étude des interactions végétaux-poissons.

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Infestations of aquatic macrophytes are common in freshwater lakes, reservoirs, and ponds throughout the world (Rottman and Anderson 1976; Thomas and Pauley 1989). In the past decade, excessive growth of aquatic plants has also become a nuisance in many locations in the Pacific Northwest (Rawson 1985). Lake Osoyoos, British Columbia, Lake Washington, Washington, and Devils Lake, Oregon, and the Columbia River and its irrigation system are just a few of the larger, more noticeable water bodies that now have large beds of aquatic plants; and hundreds of smaller water bodies are similarly affected. Management agencies with the responsibility to maintain water flow, navigation, recreational access, water quality, and fish production have begun plant control programs to reclaim the lost open-water habitat in many of these infested waters (Decell 1986).

Management agencies typically have one or two goals with regards to nuisance aquatic plants; either to eliminate, or to control them at some desired level of abundance. Several methods have proven successful for eradicating aquatic plants, but the procedures used for controlling plant biomass at a desirable level are still unproven. Confounding the development of methods to control aquatic plants is the difficulty in verification which requires accurate and precise measures of plant abundance.

Measurements of surface cover or plant biomass are commonly used to detect changes in plant abundance and examine plant control practices (Payne 1982). The surface coverage of plants is often measured using aerial photography, but surface cover underestimates plant abundance when submergent plants are present or the water transparency is low (Benton and Newman 1976). Thus, when high precision and accuracy is required for evaluating aquatic plant control practices, direct collection of macrophytes using a quadrat and SCUBA techniques are conducted to collect the data for biomass estimation (Greig-Smith 1964; Downing and Anderson 1985). Unfortunately, quadrat sampling is laborious and expensive (Downing and Anderson 1985).

Maceina and Shireman (1980) showed that measurements with a chart recording echosounder could be used to estimate

several variables related to the vertical and horizontal extent of aquatic plants in the water column: percent coverage, percent vertical cross-sectional area, mean vegetation height, vegetation volume, and standing crop. Maceina et al. (1984) developed a regression model using acoustic range measurements of plant height and signal amplitude information of plant density to estimate aquatic plant biomass in individual lakes. However, Thomas et al. (1985) found the amplitude information from plant echos to be unreliable for estimating density using the echo integration technique (Thorne 1983). Duarte (1987) eliminated the amplitude information and introduced a plant form classification parameter to account for the variability in the plant height to biomass relationship. While these authors presented innovative procedures for estimating plant biomass using acoustics, they bypassed an ecologically important variable, plant bed biovolume.

Plant bed biovolume is similar to the plant biomass variable because it is a way to describe the abundance or size of the aquatic macrophyte community. Thus, in a statistical sense, the variable chosen, biovolume or biomass, for detecting a nonspecific change to the abundance of plants in a lake should be the one that has the greatest ability to detect a change in the mean, or the most efficient estimator. Simpson et al. (1960) stated that some estimators are more efficient than others and "whenever possible the most efficient should be used."

However, both the biovolume and biomass variables have important ecological information that need to be considered for their proper application. The plant bed biovolume variable is an indicator of spatial or habitat dominance, whereas, the biomass variable is more related to absolute growth or production (Cox 1970). In many cases, lake management is primarily concerned with the amount of commercial, recreational, or habitat space that is lost to, or degraded by aquatic plants. Because of this concern, sometimes it is inappropriate to use biomass to evaluate plant control. In Devils Lake, a change in species composition of a plant community was observed to cause increases in plant biomass, despite significant reductions in the plant bed biovolume (Thomas et al. 1990). In such situations, the plant bed biovolume variable is more suitable than biomass for monitoring changes in the plant abundance. In contrast, when a manager needs to estimate a stocking rate for grass carp the plant biomass is the most appropriate variable to choose (Thomas and Pauly 1989).

Ecologically speaking, the plant bed volume variable provides an indicator of the quantity of habitat that is occupied by plants and that is free of plants (open water). The quantity of plant-occupied and open water in littoral zones of lakes must play a significant role in the production of aquatic organisms because of intra- and interspecific habitat requirements and preferences. This would especially be true if the plants exerted a significant localized impact on the quality of the plant occupied space, which they do.

Dense beds of aquatic plants partition the water column into plant and open water habitats (Rai and Munshi 1979). The plant beds have very localized abiotic and biotic effects on the littoral zone that influence the physical and chemical quality of the water (Buscemi 1958; Dale and Gillespie 1976; Rai and Munshi 1979; Cooke et al. 1986; Godshalk et al. 1986; Frodge et al. 1990), the cycling of nutrients (Barko and Smart 1980; Carpenter et al. 1983), and the distribution and productivity of organisms (Goulder 1969; Adams and Prentki 1982; Durocher et al. 1984; Wiley et al. 1984). When plant beds begin to dominate the littoral zone, the degradation of water quality can reduce the volume of habitat for production of many species (Grace and Wetzel 1978; Wiley et al. 1984).

Besides the ecological importance of the variable, we feel that a plant bed biovolume variable can be precisely estimated because it can be calculated with acoustic range information. This is supported by Duarte's observation that acoustic range estimates of plant height had low variance. Since a more precise and less expensive procedure for estimating plant abundance and distribution would be useful for verifying plant control practices and possibly for developing a better understanding of interaction of aquatic organisms and plants, we undertook this research.

The objective of this manuscript is to develop and evaluate a procedure to estimate plant bed biovolume with acoustic range information. We use independent, concurrent, random sampling designs to collect acoustic range and SCUBA quadrat measurements, calculate the precision of the plant bed biovolume and plant biomass estimators, and compare their ability to detect a change in the mean for the same sample size and for the same cost. Conquest (1983) showed that the lower the coefficient of variation (CV), the easier it is to detect a significant change in the mean, hence it is more efficient (Simpson et al. 1960). Since the biovolume and the biomass variables are on different scales, the CV is the appropriate choice of statistics for comparing the relative efficiency between the two variables (Simpson et al. 1960). The CV is consistent because it is computed from the standard deviation and the mean, both of which are consistent statistics (Simpson et al. 1960). Thus, when the CV is computed from an adequate sample size, it provides a reliable method for comparing the ability of two variables to detect change. We also conduct experiments to evaluate and reduce the limitations of acoustically measuring plant range.

Methods

Acoustical measurements of aquatic plants were made in Devils Lake, Oregon (Fig. 1) and Lake Washington, Washington. The plant beds in both Lake Washington and Devils Lake are primarily submergent species: *Myriophyllum spicatum, Ceratophyllum demersum, Egeria densa*, and *Elodea canadensis* (Thomas and Pauley 1989). Data from Devils Lake are used primarily to evaluate the statistical quality of plant survey information whereas the Lake Washington data were used primarily to evaluate the effects of acoustic system parameters on bottom detection in dense plant beds.

Devils Lake

Data acquistion

In Devils Lake, a Simrad EY-M scientific echosounder and an inexpensive commercial echosounder, a Sitex HE-203, were used to measure plants. The EY-M transmitter had a pulse power of 75 W, a frequency of 70 kHz, a pulse duration of 0.6 ms, and a source level of 104 decibels. The EY-M receiver had a 40 log r time-varied gain and a manual gain that could be increased manually by 27 db in 3 decibel steps. The nominal half-beam beam angle (at the -3 decibel pts.) of the EY-M transducer was 22°. The recorder used 15 cm dry paper and at minimum range selection (0–60 m) had a constant paper speed of 24 mm/min.

The HE-203 transmitter had a pulse power of 50 W, a frequency of 107 kHz, and a pulse duration of 0.15 ms at minimum range selection. The HE-203 receiver had 40 log r time-varied

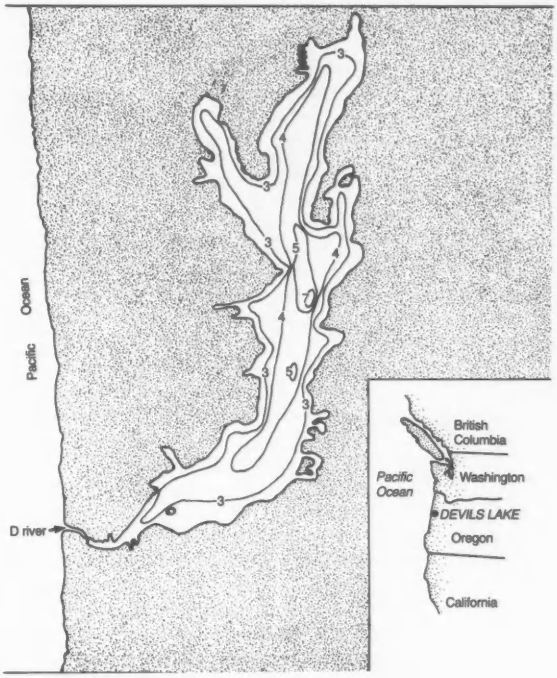

FIG. 1. Bathymetric map of Devils Lake, Oregon.

gain and a manual gain which were not calibrated in decibel steps. The nominal half beam angle of the HE-203 transducer was 25°. The recorder used 8 cm of dry paper and at minimum range selection (0–7 m) had a constant paper mark at 84-s intervals.

In Devils Lake, we conducted three acoustic surveys consisting of 14 acoustic transects each, in May, July, and September of 1986. Stratified sampling was employed: a curve was fit down the middle of the lake by eye which served as a midlake axis, the midlake axis was divided into roughly 50-m increments, and locations for transect crossings were selected randomly. The transect lines were oriented roughly perpendicular to the midlake axis at each crossing point.

The transducer was mounted on a pole that was clamped to the bow of a 5-m pram and lowered to a depth just below the surface of the water. The boat's speed was approximately 1 m/s.

Data analysis

The echogram for each transect was treated as an independent random measurement. Because the EY-M echograms were small in vertical range (360cm/cm) compared with those of the HE-203 (80cm/cm), they were photocopied and enlarged before they were processed. Version 2.5 of AUTOCAD, version 1.2 of SIGMASCAN, a MS-DOS personal computer, and a digitizing board were used to measure the vertical cross sectional areas of aquatic plants from echograms. The vertical cross section of plants was the integrated area within the envelope created by tracing the aquatic plants along a transect (the area between the top of the plants and the bottom of the water column) with a calibrated digitizing pen. The EY-M echograms were enlarged before processing and the HE-203 echograms were processed directly.

The first measurement, C_i, was of the vertical cross sectional area of the total water column (surface to bottom) along the entire transect. Bottom depths that were obscured by the plant canopy (Fig. 2) were estimated by fitting a line between the two adjacent bottom depth observations. The vertical cross sectional area was calibrated to square metres measuring transect length from a map and using the depth scale of the echogram. The vertical cross sectional area of the open water, C_{i1}, was measured in the same way. Finally, the vertical cross sectional area of the plant bed was determined by subtraction from the total water column, $C_{i2} = C_i - C_{i1}$.

The measurement of the vertical cross sectional area of plants (m^2) on each transect was assumed to be continuous, and the average height of the plants, h_i, (m) was computed exactly by dividing the C_{i2} by the transect length (m). The transect samples were Poisson, independent (did not overlap), and random, so the weighted mean and variance of the plant height could be estimated for the entire lake from the transect data (Burnham et al. 1980):

$$(1) \quad h = \sum_{i=1}^{R} h_i l_i \Big/ \sum_{i=1}^{R} l_i$$

where, h_i = average height of plants on transect i,
R = total number of transects,
and,

$$(2) \quad \text{Var } h = \sum_{i=1}^{R} l_i (h_i - h)^2 \Big/ \sum_{i=1}^{R} l_i (R-1).$$

The average height and variance of the plants were extrapolated to predict the biovolume of the plant beds by simple multiplication by the surface area (m^2) and surface area squared (m^4). The vertical and horizontal distribution of plants throughout the lake were examined using the continuous within-transect data and discrete between-transect data.

Comparison procedures

In Devils Lake, concurrent to the acoustic sampling, the aquatic macrophytes were randomly sampled using a 0.25 m^2 quadrat sampler patterned after one designed by Pukerson and Davis (1975). The May survey data was used to determine the variance of plant biomass and the sample size needed to estimate the mean biomass (g/m^2) with acceptable confidence, 15–30% (Nichols 1984; Osborne 1984; Cochran 1977). In the laboratory, the plants were sorted by species, spun to remove excess surface water, and weighed. Thomas and Pauley (1989) describe the sampling procedures in greater detail.

The CV's were used to compare the relative precision of the volume and the biomass variables since they are unaffected by the change in scale (Simpson et al. 1960). Conquest (1983) shows that the CV is a vital part of the student's-t distribution and that it can be rearranged as follows:

$$(3) \quad t = (n_i + n_j/n_i n_j)^{-1}/(s/\mu_i - \mu_j)$$

We rearrange this in terms of k, the percent of change in the mean (μ),

$$\mu_2 = \mu_1 + 100k\mu_1,$$

then by substitution it follows that,

$$t = 100k\,(n_i + n_j/n_i n_j)^{-1}/\text{CV}$$

when the CV is to be used to evaluate the ability to detect change in the mean with equal sample sizes then,

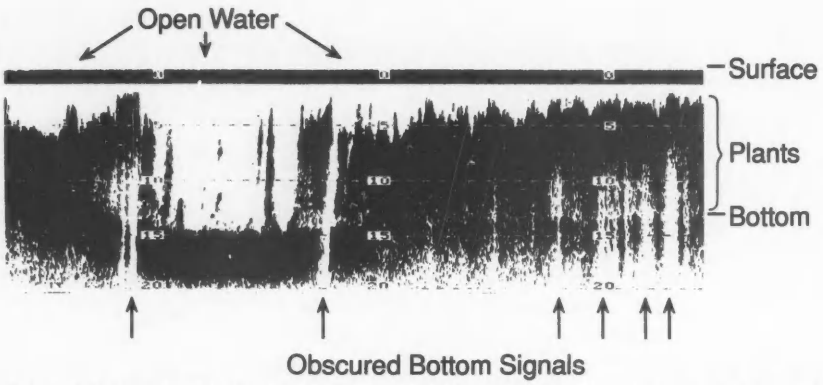

FIG. 2. Echogram showing cross sectional area of water column showing the open water and the water occupied by aquatic plants, and the areas under the plant beds where bottom detection is partially or totally obscured.

(4) $k = \text{CV}\ (t/100(n/2)^{-1} = \text{CV}\ (nt/200).$

Equation (4) allows us to compute the percentage change in the mean which can be detected with a given CV and sample size. In situations where two variables provide equivalent ecological information, one may use the ratio (k_1/k_2) as an indicator of their relative ability to detect change: for example if $k_1/k_2 = 3$ then method 2 can detect 33% of the change at the same sample size.

Furthermore, since cost (c_i) can be computed as a function of the sample size for each method, k_1 can be plotted as a function of c_i for each method, or the ratio, k_1/k_2, can be plotted in three dimensions as a function of c_1 and c_2 at $k_1/k_2 = 1$. Since 1/variance is information, we will call $1/k$ quality. In contrast to information, quality is dimensionless and can be used to compare unlike variables and the lower CV produces a lower $1/k$. Thus, the relative quality of the two methods can be established as follows,

(5) $Q_{1,2} = k_1(c_1)/k_2(c_2)$

where, $k_1 > k_2$,

$Q_{1,2}$ = the relative cost of precision of k_1 over k_2

The c_i are computed as follows:

(6) $c_i = n(L_{lk}) + E_{lk}$

where,

L = labor costs (h) (\$/h)/n,
l = cost of acquisition (\$/n),
k = cost of analysis (\$/n),
E = equipment costs (\$/36).

The following variables were averaged over the 3 yr of sampling on Devils Lake, Oregon: the time to run an acoustic transect (h), the time to collect a quadrat sample (h), and the average labor cost (\$/h). The initial cost of equipment was depreciated on a straight line scale over 3 yr (IRS form 4682), divided by 365 to yield a daily cost, and multiplied by a factor of 30 to compute a minimum lease rate (scientific equipment).

Lake Washington

Data acquistion

In Lake Washington, a BioSonics 101 scientific echosounder with a carrier frequency of 420 kHz was used to measure aquatic plants. The shortest pulse length possible (0.1 ms) and a high pulse rate (10/s) were chosen because they would provide higher resolution at the short ranges. Data were collected with six and 10 degree transducers and stored on chart paper and digital video tape using a Sony PCM-F1 digital audio processor and Sony SL-2005 beta format video cassette recorder.

The central area of Union Bay, Lake Washington, was chosen for study because it was infested with *M. spicatum*. Three areas of different plant density (100, 50, and 5%) were located by counting the number of metres along a floating, calibrated, 30-m line occupied by plants near the surface (metres of plants/30 m). A total of 132, 30-m transects were conducted to collect acoustic data, which included 22 replications in each of the six combinations of transducer and plant density. The transducers were mounted in a Braincon V fin which was towed from the bow of the boat so that it just skimmed over the surface. The boat's speed was approximately 1 m/s.

Data analysis

The Lake Washington data were played back on a Tectronics 7313 storage oscilloscope for signal analysis. Data were subsampled at a rate of 56 pulses/min since the sample rate of 10/s was too fast for manual detection of bottom signals. The percentage of pulses with bottom detection was determined for each transect, or transducer/plant density combination ($n = 132$). This statistic was used to evaluate the effect that a narrower beam pattern would have on the saturation of acoustic signals and the ability to detect bottom in plant beds.

TABLE 1. Mean $(\bar{X})$, standard deviation (SP), coefficient of variation (CV), relative statistical efficiency (k_1/k_2), and quality (Q) for plant biomass (g/m^2 spun) and plant bed biovolume (m^3) in Devil's Lake for May, July, and September 1986.

	Biomass − k_1			Volume − K_2			Efficiency	
Date	$\bar{X}$	S_D	CV	$\bar{X}$	S_D	CV	k_1/k_2	Q
5/86	1341	1309	0.98	2.75	0.49	0.18	5.44	10.34
6/86	1652	283	0.81	7.92	0.36	0.05	16.20	30.78
9/86	1367	303	1.05	7.01	0.44	0.06	17.50	33.52

Results and Discussion

Precision

The CV's for the plant biovolume were significantly less than the CV's for plant biomass ($\alpha = 0.05$), ranging from 0.05 to 0.18

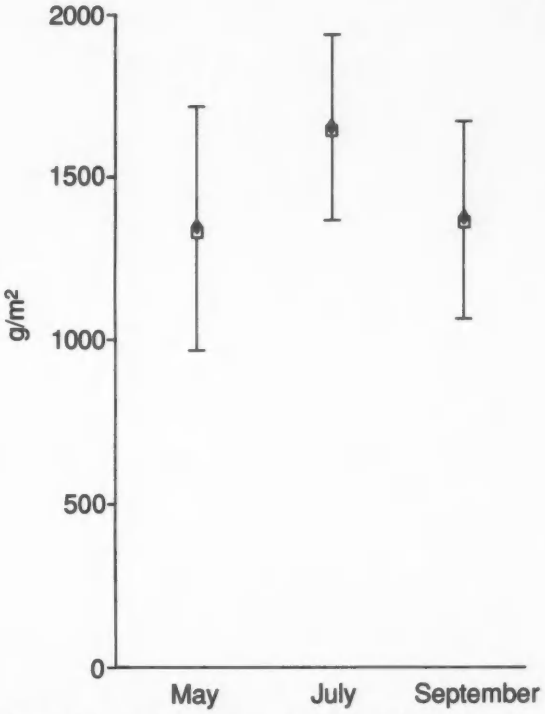

FIG. 3. Estimates of mean plant density (g/m^2) and the 95% confidence limits from three SCUBA surveys of Devils Lake: May, July, and September 1986.

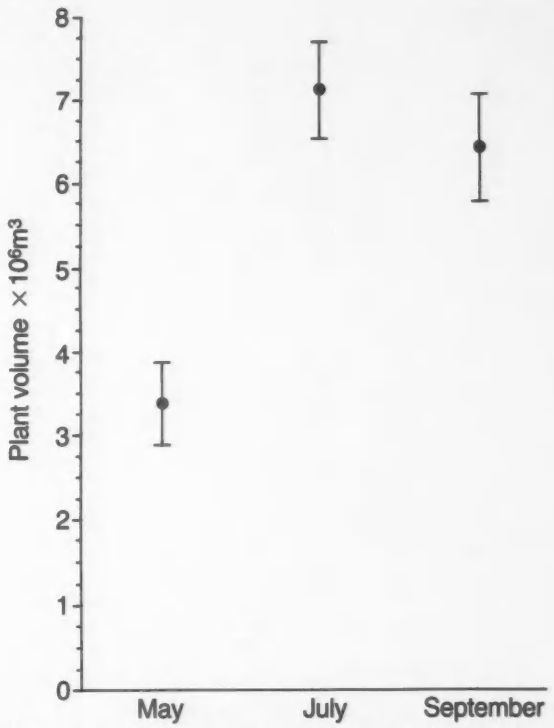

FIG. 4. Estimates of the volume of plants (m^3) and the 95% confidence limits from three acoustic surveys of Devils Lake: May, July, and September 1986.

and 0.81 to 1.05, respectively, which indicated the efficiency of the biovolume variable to detect differences in the mean was between five and 18 times greater than the biomass statistic (Table 1). For example, the mean total biomass and total biovolume of plants suggest an expected seasonal trend of summer peak biomass (Figs. 3 and 4), but only the biovolume estimates are statistically different ($\alpha = 0.05$).

Over the three years of research at Devils Lake, the quadrat sampling was 1.9 times more expensive than the acoustic sampling. The difference in costs was largely attributed to 2.5 times higher labor requirement to collect and process the quadrat samples. The equipment required to collect and process the samples was roughly the same total costs (1.08), with the major costs of the quadrat technique in the acquisition effort (SCUBA gear and sampler) versus analysis for the acoustic technique (digitizer). Thus, the precision to cost ratio of the acoustic technique ranged between 10 and 33 times that of the quadrat technique (Table 1).

The distinct advantage of quadrat sampling is the biological information provided, such as species identification and weight of the plants. Thus, the biovolume variable is not a substitution for such information, but only a supplement which is more efficient at detecting changes occurring to the spatial extent or size of a plant community. For many situations, biomass data are more appropriate or valuable, such as in the evaluation of the use of grass carp (*Ctenopharyngodon idella*), which are selective feeders, for plant control, but in other applications, such as interference with commercial, recreational, or habitat space, biovolume is more relevant.

As pointed out earlier, Maceina et al. (1984) and Duarte (1987) developed acoustic methods for estimating plant biomass. Duarte presented data that showed the aquatic plant species could be segregated into three density groups without overlap (430–1300, 1700–3000, and 3500–5500 g spun/m_3). Using the three density groups as a regression code, and the elimination of saturation level as a code (Maceina et al. 1987), Duarte developed a biomass estimator that could be used for a group of lakes. Although we found overlap in the densities for some of these plants in the Pacific Northwest (Thomas and Pauley 1989), a similar grouping was apparent during the periods of peak biomass. However, both Maceina et al. (1984) and Duarte (1987) used an "error-in-covariates structural model" to compare the acoustic and quadrat estimates, so the variance is biased (Crittenden and Thomas 1988). Although this type of analysis may suggest similar performance by the two estimators, it is not a procedure that will determine the relative or absolute precision of the techniques. As we have done for the plant bed biovolume variable, the precision of the initial measurement needs to be determined for each method independently, then compared to evaluate the quality of the estimator.

Ecological Information

Volume of habitat

The aquatic production and carrying capacity of a body of water is often related to the amount of habitat available, depth, area, or volume (Russel-Hunter 1970). When aquatic organ-

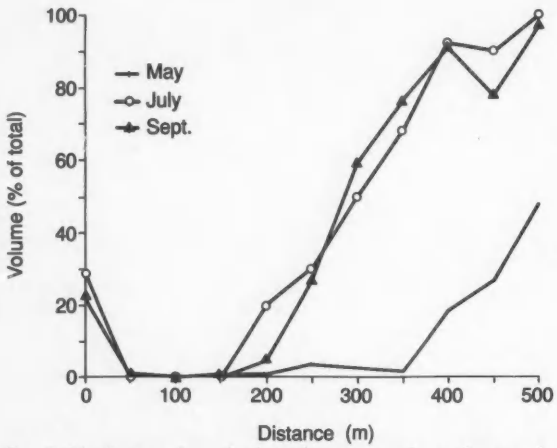

FIG. 5. Distribution of aquatic plants (by percent volume of water column) across Devils Lake in May, July, and September 1986 (Transcect 7).

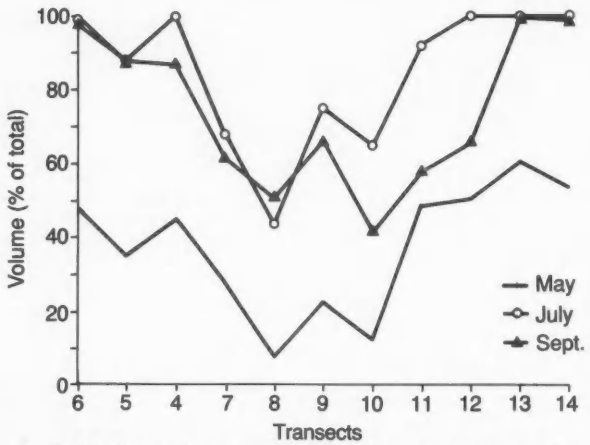

FIG. 6. Distribution of aquatic plants (by percent volume of water column) on the long axis of Devils Lake in May, July, and September 1986.

isms compete for living space there is ample evidence that growth, reproduction, and survival are affected adversely (Benf21nett 1970). The mechanisms that cause the reduced growth, reproduction, and survival are usually attributed to changes in the water quality caused by metabolism or metabolic products of the organisms themselves, such as low dissolved oxygen or a high level of ammonia (Kawamoto 1961; Rose 1959; Swingle 1956; Bennett 1970; Thomas and Pauly 1989). Frodge et al. (1990) showed this when aquatic plants infested the littoral habitat, where the effect was often localized to the water column beneath the plant bed canopies. This suggests that the volume occupied by the plant beds is probably more closely linked to the production of many littoral organisms than biomass. Thus, the development of the technique to measure plant biovolume with high precision should allow new opportunities in the evaluation of the role of aquatic plants on aquatic production.

Distribution

In Devils lake, we collected continuous data only along the short axis of the lake which was divided into 20-m divisions to extract discrete trends in plant abundance across the lake (Fig. 5). The plant distribution along the long axis was determined by treating the independent transects as discrete samples (Fig. 6).

Where plant distribution information is important for establishing spatial trends, acoustic transects should be run along the gradients of interest. For example, as a by-product of the Devils Lake surveys, the plant volume was shown to be related to the depth of the lake (Figs. 1, 5, and 6). If other gradients, such as water temperature, stream inflow, and salt water intrusion, need to be described, acoustic transects could be run along the suspected gradients.

In addition to the valuable ecological information, the knowledge of such gradients in plant distribution allows researchers to proportionally allocate discrete sampling efforts of quadrats or other sampling procedures with high precision (Cochran 1977). If weight or species information is required, the acoustic measurements of gradients in the plant distribution should be used to design the sampling plan, which will often be to stratify discrete sampling efforts proportionally with the abundance of the plants.

Sampling Improvements

The literature on underwater acoustics clearly describes the stringent requirements of transceiver quality for measuring backscattering strength (Urick 1975; Clay and Medwin 1977; Mitson 1983; Thorne 1983). Thomas et al. (1985) used scientific quality equipment to evaluate if plant biomass could be obtained by echo-integration procedures and concluded that the system parameters critical for using echo strength data to estimate plant biomass (beam width, frequency, pulse length) were not available. As an alternative approach to the use of amplitude domain information, we examined range measurements for their potential contribution.

Unlike amplitude domain data, range information is easily calibrated and can be highly accurate without the concerns of having relatively stable and linear electronics. The range from the water surface to the plant tops and to bottom substrate are the only acoustic data needed for the estimation of plant height and volume, and these data are easily stored as echograms on chart paper. Because of the dependence on echograms for data storage and retrieval, we found that the size and range of the chart record was the most important characteristic of the system. Fortunately, shallow range (0–7 m) chart recorders are standard on many low cost (less than $400) commercial echosounders, so the data acquistion equipment costs are relatively low with respect to fisheries acoustics assessments, which makes this procedure relatively nontechnical and very cost effective.

Despite the effectiveness of this technique, there are some distinct limitations. They are the detection of plants at or very near the surface, and the detection of bottom in high plant densities. Every echosounder and transducer deployment needs to be calibrated for its closest range of detection capability. In general, echosounders are not designed to make measurements within a metre of the transducer and some have displays that blank out the acoustic signal at short ranges. Also, where there are surface canopies that acoustic technique needs to be combined with a surface plant mapping procedure, such as aerial photography.

The detection of bottom in plant beds is a problem with high plant densities that can be partially solved by proper selection

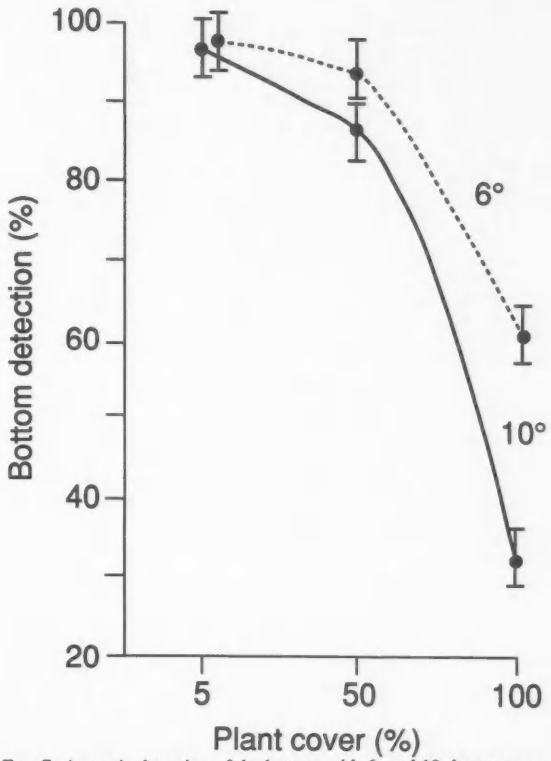

FIG. 7. Acoustic detection of the bottom with 6- and 10-degree transducers at three different densities of Eurasian watermilfoil in Lake Washington, 1984.

of manual gain and transducer-beam width. The Eurasian water milfoil bed in Union Bay of Lake Washington is very similar to most lake littoral areas in that it forms an intermittent to solid canopy. This area of solid canopy has extremely high target strength which consequently caused the plant signals to saturate the receiver, as occurred in Maceina et al. (1984) and Stent and Hanley (1985). Durate (1987) stated that he turned up the power of the acoustic signal in order to detect bottom in the denser plant growths, but the manual gain of the echosounder he described only affects the receiver gain, which presumably was already saturated. In our work on Lake Washington, we found that the lowest transmit power (−13 decibels) and receiving gain (−20 decibels) settings allowed for the most consistent detection of bottom in the dense plant beds.

Since the inexpensive commercial sounders normally do not have the capability to reduce the transmit power when saturation occurs, we explored the use of narrower beam patterns to improve bottom detection. Of the two transducers we evaluated (6 and 10 degrees), the 6-degree transducer's efficiency was significantly higher than the 10-degree transducer at detecting bottom in the intermittent and dense plant canopies, and the difference betwen the two efficiencies increased with plant density (Fig. 7). Even so, with high plant densities the six-degree transducer efficiency was only 65% suggesting that narrower beam patterns such as 1 and 2 degrees would probably be even more efficient. Given that most inexpensive echosounders are equipped with relatively wide angle transducers (20 to 40 degrees) to increase their sample volume in shallow water, we feel that the most significant modification that could be made to the inexpensive echosounders would be to replace the stock transducer with a narrower beam transducer.

Acknowledgments

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Seasonal Variations in the Smolt Characteristics of Juvenile Atlantic Salmon (*Salmo salar*) from Estuarine and Riverine Environments

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Cunjak, R. A., R. L. Saunders, and E. M. P. Chadwick. 1990. Seasonal variations in the smolt characteristics of juvenile Atlantic salmon (*Salmo salar*) from estuarine and riverine environments. Can. J. Fish. Aquat. Sci. 47: 813–820.

Smolt characteristics such as lipid-moisture dynamics, ATPase activity, salinity tolerance, and condition factor were determined for Atlantic salmon parr (*Salmo salar*) moving to, or residing in, estuaries of two rivers in eastern Canada. Lipid and water content and gill Na^+K^+-ATPase activity of these parr differed markedly from river-resident parr. ATPase activity, sex ratio, and condition factor indicated more similarity between estuarine parr and smolts. Estuarine parr were unable to tolerate high salinities in spring, summer, or autumn, but there was an indication of an autumnal increase in salinity tolerance which coincided with maximum levels in ATPase activity and a secondary downstream movement of parr and smolt-like salmon in the autumn. The data suggest that the largest estuarine parr are essentially presmolts which utilize the estuary as a rearing environment prior to subsequent emigration seaward when environmental conditions are again amenable to a reopening of the 'smolt window.' These observations also highlight some inadequacies in applying existing criteria for the smolting process and for modelling life histories of salmonids.

Nous avons étudié certaines caractéristiques des smolts comme le métabolisme des lipides et de l'eau, l'activité de l'ATPase, la tolérance à la salinité et le coefficient de condition chez des tacons du saumon de l'Atlantique (*Salmo salar*) migrant vers les estuaires de deux rivières de l'est du Canada ou y résidant. Ces tacons et les tacons vivant en rivière ont des contenus en eau et en lipides nettement différents. Il en va de même de l'activité de la Na^+K^+-ATPase au niveau des branchies. L'activité de l'ATPase, le rapport des sexes et le coefficient de conditon étaient davantage similaires chez les tacons estuariens et les smolts. Les tacons estuariens ne pouvaient tolérer les fortes salinités au printemps, à l'été ou à l'automne mais cette tolérance semblait plus grande à l'automne, période où les taux d'activité de l'ATPase étaient les plus élevés et où il y a eu une deuxième migration vers l'aval de tacons et de saumons montrant des caractéristiques de smolts. Nos données laissent entendre que les plus gros tacons estuariens sont en fait des saumons au stade prémolt qui viennent poursuivre leur croissance dans l'estuaire avant de se diriger vers la mer quand les conditions environnementales redeviennent favorables au passage des smolts dans la mer. Ces observations mettent également en évidence certaines inexactitudes dans les critères utilisés pour décrire la smoltification et modéliser le cycle vital des salmonidés.

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Cunjak et al. (1989) documented the downstream movements and subsequent estuarine residence of Atlantic salmon (*Salmo salar*) parr in a river, Western Arm Brook, in northwestern Newfoundland. Their study showed that whereas smolts emigrated within days of entering brackish water, parr, which also entered the estuary, were present throughout the summer and autumn. Further, there was evidence to suggest that the largest parr in the estuary moved seaward, either in the late spring (coincident with, or soon after, downstream migration) or in the autumn together with a secondary downstream movement of parr and smolt-like salmon. If this is the case, then we should expect to find physiological evidence as well as general biological characteristics to support our contention that these (i.e. the largest) parr are essentially presmolts which move seaward in the same year that they enter the estuary.

Thus, our objective was to determine whether Atlantic salmon parr moving towards, or residing in, estuaries displayed smolt-like characteristics by comparing them with 'true' smolts and riverine parr. Because salinity tolerance and parr–smolt transformation are seasonal phenomena (Johnston and Saunders 1981; Langdon and Thorpe 1985; Saunders et al. 1990), a secondary objective was to document any seasonal changes in physiological parameters among the different groups of salmon. A variety of tests and parameters have been used as indicators of smolt status in salmonids (see reviews by Hoar 1976; Folmar

and Dickhoff 1980; Wedemeyer et al. 1980; McCormick and Saunders 1987). Specifically, we chose to study gill Na^+K^+-ATPase activity and lipid–moisture content (Farmer et al. 1978; Saunders and Henderson 1978; Johnston and Saunders 1981; Langdon and Thorpe 1985) as evidence of physiological changes associated with smolting, and salinity-tolerance tests (Saunders et al. 1985) as a test of the ability to tolerate full strength seawater. Condition factor, sex ratio, and maturity were also determined because these factors, in association with the preceding parameters, can be used as general indicators of smolt status (Saunders and Henderson 1970; Farmer et al. 1978; Eriksson et al. 1987; Rodgers et al. 1987).

Materials and Methods

Two river systems were studied, Western Arm Brook (WAB) and South River (SR). WAB is a fourth-order river in Newfoundland (51°11′N, 56°46′W) with a drainage basin of 150 km^2. A fish-counting fence, located near the mouth of the river has been operated (May–October) since 1971. The sampling regimen for the collection of biological data on all salmon and other species moving through the fence was outlined in Chadwick et al. (1985). The estuary of WAB is a shallow delta (total area = 65 ha) with an average summer tidal fluctuation of 1.1–1.4 m. Mean salinities in the estuary ranged from 1–24‰ during our study; water temperature seldom reaches 20°C. A more detailed description of the estuary and sampling locations was given by Cunjak et al. (1989).

SR is a fourth-order river located in Nova Scotia (45°36′N, 61°55′W) which drains an area of approximately 200 km^2. A fish counting fence, located approximately 2 km above the head of tide has been used to monitor upstream and downstream movements of fishes (Chadwick et al. 1985) from spring to autumn, 1981–87. The SR estuary (79 ha) forms the southern portion of a large lagoon (Antigonish Harbour), which experiences maximum summer temperatures ≥25°C during July and August. Salinities measured during 1987 ranged from 0 to 19‰ depending on the tide and location within the estuary.

Atlantic salmon were separated into one of four groups for comparison: smolts (or smolt-like salmon), based on phenotypic characteristics (silvery body colour, absence of parr marks, darkened fins, and streamlined body shape, see Wedemeyer et al. 1980), and captured at the counting fence during their downstream movement; 'migrant' parr, which were clearly distinguished from smolts (Cunjak et al. 1989) and also captured at the counting fence during their downstream movement (WAB only); 'riverine' parr, collected by electrofishing, 0.2–0.5 km upstream of the counting fences of both rivers and assumed to represent river-resident salmon parr; and, 'estuarine' parr (formerly 'migrant' parr), which were seined from brackish water sites.

At SR, estuarine parr were collected exclusively from a single site in the upper estuary (salinity = 0–5.0) where salmon parr were most abundant (R. A. Cunjak, unpubl. data). Estuarine parr from WAB were collected from any of four sites throughout the estuary, although most (75%) were collected at a site near the head of the estuary (0–14 ppt).

At WAB, salmon were collected in both 1987 and 1988, and depending on the date of capture, samples were divided into one of four seasonal categories: spring (late May), late spring (early June, 7–12 days after 'spring' collection), summer (August), and autumn (late September). Because downstream movement of parr (i.e. 'migrants') and smolts had essentially ceased by late June, these two groups of salmon were absent from summer collections. At SR, where sampling was carried out in 1987 only, salmon parr were rare in the estuary by midsummer (R. A. Cunjak, unpubl. data) and consequently, only spring (18–22 May) and late spring (12 June) collections were made.

All the salmon collected for this study were measured (fork length, centimetres), weighed (wet weight, grams) and dissected for determination of sex and stage of maturity. Carcasses were frozen for subsequent determination of lipid and water content. Additional sampling data of parr and smolts (R. A. Cunjak, unpubl. data) from the different locations at WAB (1987 and 1988) and SR (1987) were also included for determinations of condition factor and sex ratio.

In the field, gill filaments of salmon were trimmed from gill arches, placed in 1.0 mL of SEI buffer (0.3 M sucrose, 0.02 M disodium ethylenediaminetetraacetate, and 0.1 M imidazole at pH 7.0), and immediately frozen in dry ice until transport to the laboratory and storage at −80°C. Gill Na^+K^+-ATPase activity was determined according to the method of Zaugg (1982a) and incorporating the modifications of McCormick et al. (1987). Enzyme activity was expressed as μmoles inorganic phosphate per milligram protein per hour ($\mu M\ PO_4 \cdot mg\ protein^{-1} \cdot h^{-1}$).

Whole frozen salmon were freeze-dried in a lyophilizer for 48 h at −75°C and reweighed to calculate water content. Lipid content was determined, in duplicate, from samples of whole fish that had been homogenized and freeze-dried using the methanol:chloroform extraction technique (Bligh and Dyer 1959). Values for lipid and water content were expressed as percentages of the whole wet weight of each fish.

Salinity-tolerance tests (Saunders et al. 1985) were conducted at Western Arm Brook during 1988 (spring, summer, and autumn). Ten salmon sampled from a single location (counting fence, river, or estuary) were introduced to an opaque, 91-L polypropylene container of seawater supplemented with 'Instant Ocean,' such that salinity = 37.5. A salinity of 37.5 was chosen to better test osmoregulatory development and to overcome the confounding factor of survival by some (large) parr at normal seawater concentrations (Wedemeyer et al. 1980). Containers were covered, aerated continuously, and set in the river adjacent to the counting fence for the duration of the tests (48 h). Paired comparisons of salinity tolerance were carried out as follows: in spring, migrant parr versus smolts (May 28–30), riverine parr versus estuarine parr (June 1–3); in summer, riverine parr versus estuarine parr (August 18–19); and in autumn, riverine parr versus estuarine parr (September 25–26). For each comparison, fish were matched as closely as possible for size. Containers were checked every 2 h for mortalities which were subsequently removed, measured, weighed, and frozen. Salmon from these tests were not used in the physiological determinations or for the calculation of condition factor.

Linear regression was used to assess the relationship of fish size on ATPase activity and on survival time of salmon used in the salinity-tolerance tests. Student's *t*-tests were used to check for inter-year differences of each of the physiological parameters measured at WAB. ANOVA (or Kruskal Wallis test) was used to test for inter-seasonal differences of the lipid (and water) content and for differences in mean condition factor among the different sampling locations; a posteriori SNK tests were used to identify where significant differences occurred among the class levels. Kolmogorov-Smirnov tests were used to compare

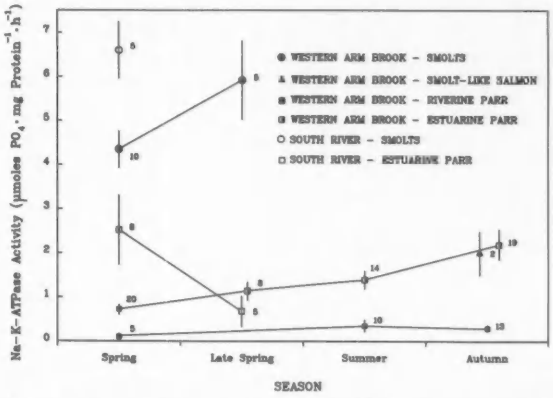

FIG. 1. Seasonal changes in mean gill Na^+K^+-ATPase activity in Atlantic salmon parr and smolts sampled from different locations in the Western Arm Brook and South River systems. Vertical bars represent the standard errors; numbers denote sample sizes.

the distributions of cumulative mortalities for paired comparisons in the salinity tolerance tests.

Results

Gill Na^+K^+-ATPase activity differed depending on the life stage of the salmon and the site and season of sampling (Fig. 1). No significant inter-year differences ($p>0.05$) were noted for ATPase activity (WAB) and consequently data from the two years were pooled. Gill ATPase activity was highest for smolts, particularly at the peak of their downstream movements (spring, SR; late spring, WAB) when activity ranged between 4.2–8.0 (SR) and 3.9–8.3 (WAB). Riverine parr from WAB had negligible Na^+K^+-ATPase activity (0–0.8) regardless of the season (Fig. 1); gill tissue for SR riverine parr was not analysed. Mean gill ATPase activity for migrant salmon parr sampled at the counting fence during their downstream movements did not differ significantly ($p>0.70$) from that of estuarine parr, when they were sampled concurrently in spring and late spring. Therefore, these data were pooled and will be collectively referred to as estuarine parr (Fig. 1). However, estuarine parr had significantly higher ($p<0.02$) Na^+K^+-ATPase activity than river-resident parr in each of the spring, summer, and autumn sampling periods (Fig. 1). Despite the increased enzyme activity of the estuarine parr, mean levels were considerably less than those measured for smolts, at both WAB and SR (Fig. 1). However, 23 and 8% of estuarine parr from SR and WAB, respectively, had Na^+K^+-ATPase activity within the range measured for smolts during the peak of their migration. These high activities for estuarine parr from WAB were recorded exclusively in the autumn.

There was an increase in Na^+K^+-ATPase activity from spring (range = 0–1.5) to autumn (0.2–5.7) for estuarine parr from WAB (Fig. 1). However, the increase between successive sampling dates was significant ($p<0.01$) only between summer and autumn when gill ATPase activity levels were maximal. This higher ATPase activity of parr was nearly identical to the measured enzyme activity of smolt-like salmon in the autumn (Fig. 1), and coincided in time with a secondary downstream movement by juvenile salmon. These autumnal movements (i.e. after 1 September) have varied in magnitude in recent years

TABLE 1. Mean sizes (FL, cm ± SD) of different life stages of Atlantic salmon sampled for physiological parameters at various sites at Western Arm Brook (WAB), Newfoundland (1987 and 1988 data pooled) and South River (SR), Nova Scotia. Values in parentheses are the range of FL measured; dashed values indicate that no sample was collected. Values in square brackets are sample sizes.

Location	Life stage	Collection sites: River	Counting fence	Estuary
WAB	Parr	10.8 ± 1.94[41] (7.8–16.0)	12.5 ± 1.01[22] (10.3–13.7)	10.6 ± 1.78[45] (7.2–15.0)
	Smolts	18.9 ± 1.86[3] (16.8–20.4)	16.8 ± 2.10[21] (12.9–21.0)	18.2[1]
SR	Parr	6.9 ± 0.97[6] (5.3–8.0)	—	8.3 ± 1.53[17] (6.3–11.0)
	Smolts	—	12.5 ± 0.87[5] (11.3–13.5)	—

from 140 parr and 53 smolt-like salmon in 1983 to as few as 54 parr and six smolt-like salmon in 1988 (Cunjak et al. 1989; E. M. P. Chadwick, unpubl. data). Mean sizes (FL) of parr during this period (11.7–12.9 cm) tend to be slightly greater than the annual means for parr migrants (9.9–11.9 cm) but significantly smaller than the size of smolt-like salmon in autumn (15.5–20.4 cm).

An anomalous trend was noted for SR estuarine parr. Gill Na^+K^+-ATPase activity decreased (Fig. 1) between spring (0.1–6.0) and late spring (0–1.9). This corresponded with a marked decline in mean size (FL ± SD) for parr sampled in spring (9.3 cm ± 1.6) compared with late spring (7.3 cm ± 0.2). At SR, large parr emigrate from the estuary (presumably seaward) in the spring (R. A. Cunjak, unpubl. data) coincident with the peak in the smolt migration.

Because size is known to influence salinity tolerance and the parr–smolt transformation (e.g. McCormick and Saunders 1987), we investigated its effect on Na^+K^+-ATPase activity. Smolts from both river systems were markedly larger than parr, regardless of sampling location (Table 1) and this may have contributed to the high enzyme activity measured for smolts. Parr sizes were comparable (Table 1) among sampling sites at SR, and at WAB. There was a significant, positive effect of estuarine parr size (FL) on Na^+K^+-ATPase activity at both SR ($p<0.001$) and WAB ($p<0.05$). Size could explain 79% of the variation at SR where it was noted (see above) that parr size in the estuary changed markedly between the two sampling periods; at WAB, only 10% of the variation in gill ATPase activity for estuarine parr was explained by size. Size had no obvious influence ($p>0.45$) on enzyme activity of riverine parr and explained <2% of the variation in these data. Although water temperature and salinity varied with the date and site of sampling, we detected no influence of these parameters on gill ATPase activity during this study. Sex also had no significant effect ($p>0.05$) on gill ATPase activity of parr (or smolts) from either riverine or estuarine locations at WAB and SR.

Mean lipid levels were generally lowest for smolts (4.1%, SR; 2.5–3.6%, WAB) and declined between spring and late spring as was the case for estuarine parr but unlike the trend for riverine parr wherein lipid content increased slightly (Fig. 2). Lipid and water content of estuarine and riverine parr were similar ($p<0.10$) in spring, but deviated significantly ($p<0.05$) thereafter (Fig. 2). By autumn, the mean lipid content of descending smolt-like salmon (2.5%) and estuarine parr (2.8%) were similarly low; both had significantly lower ($p<0.05$) lipid

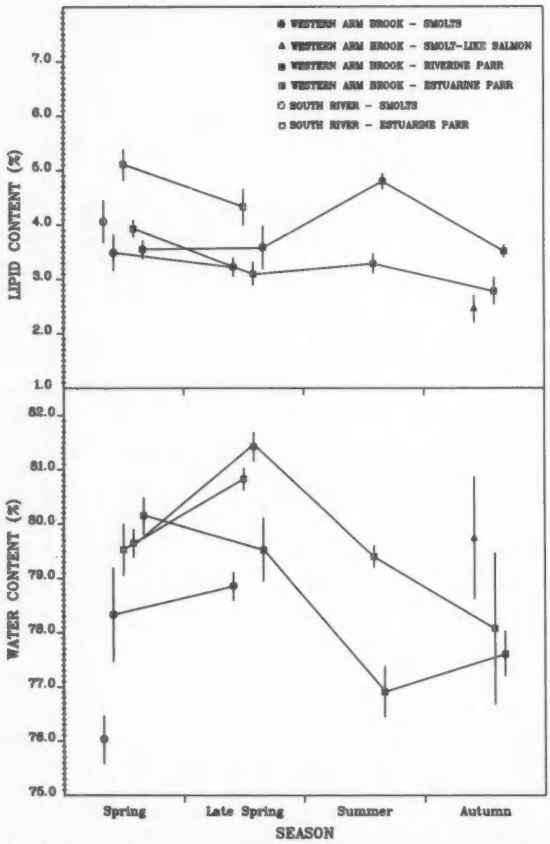

FIG. 2. Seasonal changes in mean 'whole body' lipid content (upper panel) and water content (lower panel) of Atlantic salmon parr and smolts sampled from different locations in the Western Arm Brook and South River systems. Vertical bars represent the standard errors. Sample sizes are nearly identical to those shown in Fig. 1.

contents than riverine parr (3.5%). Although mean water content in the autumn indicated less similarity between smolt-like salmon and estuarine parr than was the case for lipid content (Fig. 2), at least some of these parr (seven of 20 sampled) had an equally high water content whereas only one of the riverine parr had a body water content as high as that found for the smolt-like salmon.

The salinity-tolerance tests indicated that smolts had very high survival for the duration of the spring experiment, whereas only one of the 10 parr captured at the counting fence survived the full 48 h (Fig. 3). In the other three experiments where riverine parr were tested against parr from the estuary, none of the parr survived to the end of the test, although in spring and especially in the autumn, estuarine parr did survive the longest (Fig. 3). Water temperatures recorded during the spring and autumn tests were similar, averaging 9–10°C. In the summer, water temperatures ranged between 14 and 17°C and may have contributed to the rapid demise of both riverine and estuarine parr, when no parr survived beyond 18 h (Fig. 3).

The effect of parr size on survival in the salinity-tolerance tests was also investigated. For both estuarine (including migrants) and riverine parr, there was a significant ($p<0.05$) and positive effect, but the regressions explained only 11–12% of the variation in the survival times. Parr sizes (FL) ranged between 6.5 and 12.9 cm for the riverine versus estuarine comparisons; for the migrant parr versus smolts comparison (Fig. 3), sizes varied from 11.1–18.5 cm and 13.7–16.4 cm, respectively.

Salinity tolerance of estuarine parr increased from spring to autumn (Fig. 3). Riverine parr had essentially the same survival times in all seasons. Estuarine parr, however, which were less tolerant ($p<0.001$) of the high salinities in the spring were more tolerant ($p<0.001$) than riverine parr by autumn. We found no evidence that parr sampled from different locations in the estuary had better (or worse) survival in these tests. All fish were immature and sex had no obvious influence on the results.

Condition factor (K) of salmon was highest for parr resident in the river and lowest for smolts (Table 2). This was the case for both river systems and for both years of sampling. Mean condition factor of parr captured at the fence (i.e. migrants) and from the estuary were not significantly different ($p>0.05$), although values did decline in the estuary (Table 2). Seasonal variations in mean K suggested similarities between smolts and estuarine parr, especially during the spring and late-spring, times which corresponded to peaks in smolt migration at SR and WAB, respectively. During these periods, mean K of estuarine parr was least (SR, 1.00; WAB, 0.82) and closely resembled the condition factor of smolts (Table 2).

At WAB, females comprised 76.2% of the smolts sampled, similar to the percentages of female parr collected at the counting fence and in the estuary (Table 3). At SR, there was also a strong bias towards females among smolts; parr sampled from the estuary of SR had a lower percentage of females compared with WAB, but they were more common than males in the population (Table 3). Among the riverine parr populations of both WAB and SR, males were clearly more abundant (Table 3). Further, among male parr captured in the estuary of SR or WAB in 1987 and 1988, there was no evidence of gonadal maturation. This was in marked contrast with male parr captured in the river. For example, at WAB, 65% of the male riverine parr were identified as sexually mature or maturing.

Discussion

The results of this study support our contention that many of the Atlantic salmon parr utilizing the estuaries of Western Arm Brook and South River are essentially presmolts. Elevated gill Na^+K^+-ATPase activity coincident with smolt migration from the rivers, similar condition factors, sex ratios, and changes in body water and lipid content attest to the similarity of estuarine parr and smolts while contrasting with parr residing in the rivers. We are not, however, suggesting that all of the parr residing in estuaries are physiologically adapted for osmoregulation in seawater soon after entering brackish water. This scenario is likely only for those smolt-sized parr entering the estuary in early spring which are in a transition phase with respect to smolt-like characteristics. Indeed, our data for WAB suggest that many of the parr which emigrate (seaward?) from the estuary, do so in the autumn whereas smaller parr move back upstream to overwinter in the river and subsequently re-enter the estuary the following spring as parr, or as smolts (Cunjak et al. 1989).

Because increased gill Na^+K^+-ATPase activity is considered a necessary prerequisite to saltwater adaptation (Zaugg and Wagner 1973; Zaugg 1982b; Langdon and Thorpe 1985;

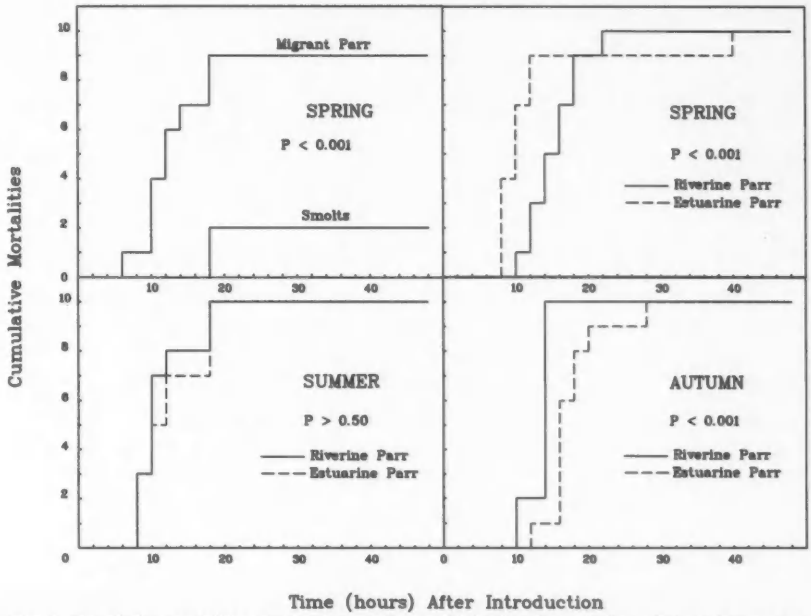

FIG. 3. Cumulative mortalities of paired comparisons of Atlantic salmon during salinity-tolerance tests (salinity = 37.5) at Western Arm Brook during the spring (May 28–June 3), summer (August 18–19), and autumn (September 25–26). Statistical significance refers to the results of Kolmogorov-Smirnov tests comparing the distributions of cumulative mortalities for each paired comparison shown in the panel.

TABLE 2. Mean condition factor ± SD of Atlantic salmon parr and smolts sampled from different sites in the South River (SR) and Western Arm Brook (WAB) systems in 1987 and in 1988. Values in parentheses are sample sizes. Horizontal lines beneath mean values indicate nonsignificant differences ($p > 0.05$) of K of parr between sampling sites or in comparison with smolts (captured at the counting fences.

		Sampling sites – Parr			
Year	Location	River	Counting fence	Estuary	Smolts
1987	WAB	1.16±0.139 (40)	0.97±0.105 (245)	0.95±0.111 (67)	0.83±0.103 (205)
	SR	1.14±0.080 (14)	—[a]	1.05±0.081 (41)	0.97±0.060 (25)
1988	WAB	1.10±0.106 (314)	1.00±0.115 (205)	0.98±0.084 (246)	0.86±0.091 (22)

[a]No parr were measured at the SR counting fence.

McCormick and Saunders 1987), the intermediate mean values measured for estuarine parr (relative to smolts and riverine parr) suggested that some of these parr were experiencing physiological changes indicative of the smolting process. Parr captured at the counting fence enroute to the estuary displayed elevated gill ATPase activity similar to parr already in the estuary. This would seem to dispel the possibility that exposure to saltwater alone (e.g. Epstein et al. 1967; McCormick and Saunders 1987) was responsible for the levels measured in this study. Such enzymatic activity during downstream movements and prior to seawater entry is well known for smolts of Atlantic salmon and other species (Zaugg and McLain 1970; Saunders and Henderson 1978; Buckman and Ewing 1982; see McCormick and Saunders 1987 for review).

Seasonal changes in enzyme activity varied between the two rivers and may reflect responses to contrasting environments. At South River, estuarine parr showed greatest Na^+K^+-ATPase activity in the spring coincident with the peak of the smolt migration (mid-May). By late spring, mean size of parr and enzyme activity were reduced. We infer from these data a seaward movement, by the largest parr (i.e. presmolts), early in the spring. In support, R. A. Cunjak (unpubl. data) captured two smolts in the estuary of SR on May 22, 1987 which had been marked at the same site 1 wk earlier, but called 'estuarine parr'; no other salmon originally marked and identified as parr in the estuary were recaptured on this date. The estuary of South River warms quickly and by July water temperature often exceeds 25°C. This may preclude a protracted estuarine resi-

TABLE 3. Percentage representation by female Atlantic salmon subsampled from the estuary, counting fence (during downstream migration) and in the river at Western Arm Brook (WAB) and South River (SR). Data are pooled for 1987 and 1988. Values in parentheses are the total sample sizes used for sexual determination.

		Site of collection		
Location	Life stage	Estuary	Counting fence	River
WAB	Parr	71.8 (156)	64.4 (104)	38.0 (108)
	Smolts	—	76.2 (239)	—
SR	Parr	58.5 (41)	—[a]	23.1 (13)
	Smolts	—	77.5 (40)	—

[a]No parr were sampled at the counting fence in South River.

dence by Atlantic salmon parr which prefer lower temperatures (Javaid and Anderson 1967; Peterson et al. 1979) such as those found at sea or in parts of the lower river (R. A. Cunjak, unpubl. data). Power and Shooner (1966) found juvenile salmon from May to August in the estuary of the Nabisipi River, Quebec, where water temperatures remained below 20°C. Semi-monthly seining collections in the South River estuary (R. A. Cunjak, unpubl. data) yielded fewer and smaller parr after June. In the estuary of Western Arm Brook, water temperature rarely exceeded 20°C, and parr were found in relative abundance from spring to autumn (Cunjak et al. 1989).

Unlike the situation at South River, estuarine parr from Western Arm Brook showed maximal gill Na^+K^+-ATPase activity in the autumn, similar to that measured for smolt-like salmon during a secondary downstream movement of juvenile salmon in the autumn (Cunjak et al. 1989). However, gill Na^+K^+-ATPase activity of WAB smolt-like salmon (identification based on phenotype) in the autumn was lower than that measured for smolts (WAB and SR) in spring or late spring. Saunders et al. (1990) and Suanders and Harmon (1990) found similarly low ATPase activity in the autumn in smolt-sized parr reared in the laboratory although salinity tolerance was high. Recent evidence (Gracey 1989) from the aquaculture industry has shown that large Atlantic salmon parr (>70 g) could be transferred directly from freshwater to saltwater cages in November with resultant low mortality (10–20%). These findings may indicate a need to redefine our criteria for smolting with respect to the seasonal relationship between salinity tolerance and physiological parameters.

Our results suggest that the "smolt window" may be reopened in the autumn when environmental conditions (e.g. water temperature, daylength) are similar to the spring and that the term "autumn smolt" may be appropriate not only for WAB, but also for other populations of *S. salar*. The autumnal migration of smolt-like Atlantic salmon has been documented from rivers in North America (Meister 1962; Power 1969; Hutchings 1986) and Europe (Youngson et al. 1983). Reimers (1973) noted the phenomenon for juvenile chinook salmon (*Oncorhynchus tshawytscha*) residing in the estuary of an Oregon river and Ewing et al. (1980) correlated autumnal peaks (which were lower than spring peaks) in Na^+K^+-ATPase activity with seawater entry of juvenile chinook salmon. At WAB, phenotypic evidence of smolting in autumn was provided by the recapture of two smolt-like salmon in the estuary in mid-September of 1987 which were previously marked as parr in the estuary in May and August (R. A. Cunjak, unpubl. data); no other recaptures of smolts (bearing marks given when they were identified as 'estuarine parr') were found on this or previous dates, although parr were abundant.

The inability of estuarine parr to survive the salinity-tolerance tests indicated that their osmoregulatory abilities were not fully developed for immediate entry to seawater, unlike the case for most of the smolts. Much of this may be explained by the small size of the parr used in the experiments. The critical size for smolting of Atlantic salmon is considered to be approximately 12 cm FL (Parry 1960; Farmer et al. 1978; Wedemeyer et al. 1980). In our experiments, only two of the 30 estuarine parr were >12 cm FL, and it was these individuals which had the longest survival times (28 and 40 h). Larger parr were scarce in the estuary, and we hypothesized (R. A. Cunjak et al. 1989) that they emigrated seaward, either in the spring or autumn, after attainment of a critical size for smolting because they continue growing while in the estuary (Power and Shooner 1966; R. A. Cunjak, unpubl. data). McCormick et al. (1985) considered the protracted estuarine residence of brook trout (*Salvelinus fontinalis*) from Quebec rivers was a means of acclimating to saltwater especially if size and species-specific, osmoregulatory physiology were inadequate to accommodate an immediate entry to the sea.

The results of the tests did suggest that salinity tolerance in estuarine parr showed a seasonal trend with best survival occurring in the autumn. This further supports our contention of an autumnal "smolt window" utilized by larger estuarine parr. The estuary, with its variable but intermediate salinites may provide a suitable environment for the development of salinity tolerance. Tschaplinski (1988), in studying the use of the Carnation Creek estuary in British Columbia by coho salmon (*Oncorhynchus kisutch*), noted an increase in salinity tolerance from May until November when they emigrated seaward.

At Western Arm Brook, during the late spring and autumn when we believe seaward movements to be occurring, the lipid content of smolts, and smolt-like salmon, was low and similar to levels of at least some (i.e. larger) of the estuarine parr which we also believe emigrated at these times. Generally, total body lipid content tends to decrease (and water increase) during the parr–smolt transformation presumably due to the accelerated lipid (and protein) catabolism requisite for osmoregulatory adjustment (Blake et al. 1984) prior to entry to the sea. The low lipid levels (and condition factors) measured for estuarine parr in the summer were in contrast with those for riverine parr and did not reflect conditions in the estuarine environment where food is generally abundant (Haedrich and Hall 1976; Simenstad et al. 1982) and where feeding (and growth) rates of salmonids are often high (Power and Shooner 1966; Reimers 1973; Healey 1980; MacDonald et al. 1988). Instead, it appears that the proximate body composition of estuarine parr is indicative of the smolting process. If the hypothesis that smolting inhibits maturation (Thorpe 1982, 1987) is valid because osmoregulation is metabolically too costly to accomodate gonadal development as well, then the absence of any mature parr in the estuaries of WAB and SR (compared with the river) could well be explained on this basis.

Assuming that estuarine parr eventually emigrate seaward based on CPUE data (Cunjak et al. 1989) and physiological changes (this study), these salmon provide an interesting data set for the size-bimodality model of Atlantic salmon (Thorpe 1977, 1986). Briefly, the model suggests that the physiological decision regulating growth in parr occurs in the summer preceding smolt emigration. The 'upper mode' (UM) group maintains growth, completes smolting and emigrates the following

spring; the 'lower mode' (LM) group arrests growth and remains in the river as parr. If there are several age-classes in the population (as was the case at WAB and SR), then these classes need to be analysed separately for differentiation of modal groups. At both WAB and SR, mean sizes of estuarine parr indicated distinct differences compared with smolts. At WAB, where age-classes of smolts and estuarine parr overlap, smolts were significantly ($p<0.001$) larger-at-age (R. A. Cunjak, unpubl. data). This would suggest that estuarine parr belong in the LM group. However, their migratory behaviour and smolt-like characteristics (especially by autumn), and their younger modal age (Cunjak et al. 1989) suggest that they might also be classified with the UM group. Perhaps, these parr represent the smallest individuals within the hypothetical 'UM' group the previous autumn/winter but by spring, although emigrating, their smolt development is incomplete. The estuary may provide the necessary conditions for further growth and the development of salinity tolerance (e.g. Power and Shooner 1966; Healey 1982) for eventual emigration in autumn or the following spring (see Cunjak et al. 1989).

In the case of the "autumn smolts" at WAB, we believe that these fish were smolts the previous spring but delayed migration for some reason — perhaps an environmental constraint. In the autumn, in concert with suitably sized parr (migrants or estuarine residents), "autumn smolts" began to migrate when the temperature (and photoperiod ?) fell below a critical level. Such a scenario might explain the fate of salmon "postsmolts" documented from other boreal estuaries (e.g. Power and Shooner 1966; Randall and Power 1979). Obviously, models such as proposed by Thorpe and others (e.g. McCormick et al. 1985) require some adjustment to accomodate variable life history tactics in wild populations. Further research, such as determinations of growth rates for estuarine parr, might elucidate the picture further.

In conclusion, our data support the hypothesis that many Atlantic salmon parr residing in estuaries display characteristics indicative of the smolting process. Further, we suggest that these parr eventually migrate seaward when environmental conditions are amenable to emigration. The fate of these 'parr' and their contribution to the returning adult population remains to be determined. Recent evidence from tagging studies in the Conne River estuary in Newfoundland has shown (based on the recapture of tagged grilse ascending the river, B. Dempson, Department of Fisheries and Oceans, St. John's, Nfld, pers. comm.), that some of these parr survive at sea to return as adults. Estuarine parr may, therefore, represent an adaptive component in the Atlantic salmon populations of these river systems. The fact that they are essentially presmolts is evidenced by their similarities to smolts in important physiological indicators as well as general body condition and sex ratios. These data also highlight some of the difficulties in applying existing criteria for defining smolting in salmonids. We suggest that future definitions of smolting should reflect the species' plasticity and adaptiveness in nature.

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Assessing the Potential Extent of Damage to Inland Lakes in Eastern Canada due to Acidic Deposition. III. Predicted Impacts on Species Richness in Seven Groups of Aquatic Biota

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Minns, C. K., J. E. Moore, D. W. Schindler, and M. L. Jones. 1990. Assessing the potential extent of damage to inland lakes in eastern Canada due to acidic deposition. III. Predicted impacts on species richness in seven groups of aquatic biota. Can. J. Fish. Aquat. Sci. 47: 821–830.

Linked regional chemical and biotic models predicted that at least 20% of all lakes in 15 of 38 secondary watersheds in eastern Canada had lost at least 20% of their potential species richness given 1980 acidic sulphate deposition levels; 20% of lakes in 15 watersheds represents circa 55 000 lakes. Fish and molluscs were the most affected of seven aquatic taxonomic groups, and rotifers the least. Mean percent loss of potential richness in all of eastern Canada ranged from 5.0 to 9.5% for the seven groups of biota under 1980 acidic deposition. Sulphur dioxide emission reductions of 42% in Canada and the United States were predicted to reduce the number of affected watersheds to seven, leaving circa 25 000 lakes with 20% or more loss of potential richness. Greater acidic deposition reductions will be needed to minimize the biotic damage affecting large areas of eastern Canada.

Selon des modèles régionaux faisant le lien entre les conditions chimiques et biotiques, au moins 20 % de tous les lacs de 15 des 38 bassins hydrographiques secondaires de l'est du Canada, soit environ 55 000 lacs, ont perdu au moins 20 % de leur diversité biologique potentielle, en termes d'espèces, à cause des retombées acides sulfatées de 1980. Parmi les sept groupes taxonomiques aquatiques étudiés, les poissons et les mollusques étaient les plus touchés tandis que les rotifères étaient les moins touchés. La perte moyenne de diversité biologique potentielle due aux retombées acides de 1980 dans tout l'est du Canada variait de 5,0 à 9,5 % pour les sept groupes à l'étude. La réduction de 42 % des émissions d'anhydride sulfureux au Canada et aux États-Unis devrait faire passer le nombre de bassins touchés à sept; dans ces conditions, il y aurait encore environ 25 000 lacs dont la diversité biologique potentielle serait diminuée de 20 %. Il faudra que les retombées acides, touchant de vastes régions de l'est du Canada, diminuent davantage pour que les dommages environnementaux dont elles sont responsables soient réduits au minimum.

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Damage suffered by aquatic biota is a major consequence of lake acidification for both ecosystems and humans. Models must be used to assess this damage because acidic deposition is falling on vast areas and it is impossible to directly measure the conditions in every lake. While there are many models describing the link between acidic sulphur deposition and lake chemistry (cf. Reuss et al. 1986), there are few linking sulphur emissions and biotic damage. Predictions from models of biotic damage in conjunction with those of the chemical status of regional populations of lakes can provide measures of total impact on the resource. The resource considered here includes the more than 700 000 lakes in Canada east of the Ontario–Manitoba border and south of latitude 52 (Minns and Kelso 1986) and all the biota they contain now or contained prior to acidification. (This total number of lakes is most likely an underestimate as it is based on incomplete data — R. Helie, pers. comm., Environment Canada, Hull, Quebec). Only when the impact on the whole of this resource has been assessed, can the trade-offs between controlling the sources of acidic deposition and allowing receptor resource losses to be assessed for aquatic ecosystems. Thus, models linking cause and effect are the ecological component of a broad framework for impact assessment wherein the 'costs' (ecological, social, and economic) of controls are compared with the 'costs' of damage.

In this paper, we have used a model of biotic damage due to lake acidification developed by Schindler et al. (1989) to predict the potential magnitude and extent of damage by acidic deposition to the population of lakes in eastern Canada. The model

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is also used to predict the effects of different levels of sulphur dioxide emissions. An integrated regional model of lake acidification (DFO-ESSA) which links sulphur emissions through deposition to lake chemistry, is described in two earlier papers in this series; a chemical 'site' model for lake acidification was derived (Marmorek et al. 1990) and applied in a regional model of lakes' chemistry in eastern Canada (Jones et al. 1990). We have also assessed the regional model's sensitivity to parameter uncertainties (M. L. Jones, K. J. Heltcher, D. R. Marmorek, and C. K. Minns, unpubl. data).

The regional chemical modelling predicted an uneven distribution of chemical effects due to differences between the distributions of sensitive lakes and acidic deposition across eastern Canada (Jones et al. 1990). The degrees of chemical impact were characterized using a variety of pH and alkalinity threshold criteria. The various emission reduction scenarios did not produce a uniform response in all watersheds because of the uneven distribution of emission sources in North America. Overall, the watersheds with the highest proportion of lakes with alkalinity ≤ 0 $\mu eq \cdot L^{-1}$ or pH ≤ 5.5 were concentrated in south-central Ontario, south-eastern Quebec, and the southern Maritimes. The predictions of lake chemistry also indicated that proposed emission reductions of 42% at all Canadian and United States sources would not eliminate the problem of lake acidification in eastern Canada. In this paper, we have used the predicted chemical changes in lakes to predict the effects on lake biota.

Materials and Methods

Site Acidification Model

The derivation and analysis of the site model was described in detail by Marmorek et al. (1990). The model is based on principles of mass and charge balance applied to the prediction of alkalinity. The steady state prediction of alkalinity associated with a given level of acidic deposition ($[Alk]_\infty$) consists of two parts in the site model: predicting the original alkalinity before acidic deposition ($[Alk]_0$) and predicting the change in alkalinity due to acidic deposition ([Alk]). Acidic deposition (D_a) was defined as the sum of total wet and dry sulphate deposition less the equivalent of the base cation (calcium + magnesium + sodium + potassium) wet deposition minus wet chloride deposition (D_n). Nitrogen was not considered in the site model.

Changes in alkalinity were assumed to be the sum of three components: the acidity (negative alkalinity) expected if all the acidic deposition appeared in runoff, plus the alkalinity due to the proportion (F_w) of acidic deposition falling on the watershed that was neutralized via cation exchange processes in the soils, and the alkalinity due to the reduction of sulphate in the lake sediments. The proportion (F_L) of in-lake sulphate reduction is a function of lake flushing rate (Baker et al. 1986; Kelly et al. 1987); the higher the flushing rate, the lower the proportion of sulphate reduced in the lake.

The original alkalinity was calculated from a charge sum balance; current base cations corrected for the increase from the watershed due to acidic neutralization (F_w) less original chloride which was assumed to equal current chloride and less original sulphate which was assumed to be equivalent to base cation minus chloride deposition divided by runoff and reduced by in-lake neutralization. The watershed neutralization factor (F_w) was assumed to be a simple function of original base cations such that F_w rose linearly from zero at zero base cations concentration to one at 200 $\mu eq \cdot L^{-1}$. Above 200 $\mu eq \cdot L^{-1}$, F_w was one. The 200 $\mu eq \cdot L^{-1}$ level was chosen as a conservative figure and reduces lake acidification considerably except when the watershed to lake area ratio (r) is small ($< 5:1$).

Regional Acidification Model

Jones et al. (1990) described in detail the use of the 'site' model in the context of an integrated regional model of acidification. The site model was applied to the estimated total population of lakes in each of 38 secondary watersheds in eastern Canada (Fig. 1). The application to each watershed was achieved by deriving estimates of the distributions of original alkalinity ($[Alk]_0$), runoff (R), watershed to lake area ratio (r), and deposition (D). The alkalinity and r-ratio distributions were derived from regional surveys of lakes between 1979 and 1982 (Kelso et al. 1986). The data were assumed to be representative of all lakes by region and to represent the 'current' condition of lakes in this study. The runoff data were obtained from summaries produced by the Water Survey of Canada. The 1980 deposition fields for sulphate were provided by Atmospheric Environment Service (AES), and for base cations and chloride were obtained by the interpolation from network data provided by AES.

The distributions of model input variables were considered to be independent and for a given deposition scenario all combinations weighted by their relative frequency were used to predict the cumulative percentage distribution of steady state alkalinity (Jones et al. 1990). The alkalinity distributions were converted to pH distributions using the standard equations (Stumm and Morgan 1981) fitted to the survey data (Kelso et al. 1986). We used a pCO_2 of 2.8 to obtain the best fit between measured pH and alkalinity values (Jones et al. 1990).

The consequences of different emission control scenarios were predicted using a source–receptor model linking 1980 sulphur dioxide emissions to the observed 1980 regional pattern of acidic sulphate deposition (Olson et al. 1983). Differences between observed and predicted 1980 sulphate deposition were used to adjust the depositions predicted under other scenarios where emissions were changed. Four scenarios were considered: (1) 1980 deposition, (2) a Canada-only emission control program modelled on the existing federal–provincial agreement and equivalent to a 42% reduction at all Canadian sources from 1980 levels (Young 1988), (3) a Canada–United States emission control program equal to a 42% reduction at all sources in both countries, and (4) a worst case of 10% increase in emissions at all sources. The regional pattern of nonacidic sulphate deposition was assumed to be constant in all scenarios. The nonacidic sulphate deposition values were used to estimate original lake sulphate concentrations which were then used in conjunction with the lake survey chemistry data to estimate original alkalinity distributions.

The predictions for secondary watersheds were pooled for regions (Ontario, Quebec, and Maritimes) and all of eastern Canada using estimates of the number of lakes in each watershed as weights (Jones et al. 1990).

Potential Richness Model

From published lists of species occurrences in lakes and streams, Eilers et al. (1984) compiled lists of species' pH-minima, i.e. the minimum pH at which a species has been observed to survive in field conditions. They compiled lists for eight taxonomic groups of aquatic biota including algae (field minima

FIG. 1. Location of the 38 secondary watersheds.

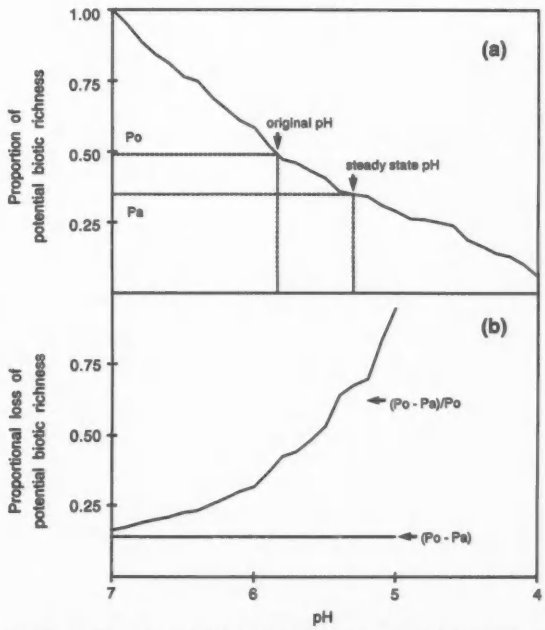

FIG. 2. An illustration of the percentage richness model showing: (a) the average response curve for seven taxonomic groups of aquatic biota and the calculation of percentage loss, $(P_0 - P_a)$ and (b) the contrast in calculated loss values with and without relative scaling.

for 264 species), rotifers (67), leeches (46), molluscs (92), crustaceans (185), insects (370), fish (66), and sponges (14). The data were obtained primarily from North American sources, particularly the north-eastern quadrant of the continent. They found that laboratory minima based on experimental data were typically about 1 pH unit lower than field values and that lake minima were usually lower than corresponding stream minima for the same or similar orders or families of biota. By taxonomic group, they graphed the proportion of species found at various pH's from 7.0 to 4.0 in 0.1 intervals; the proportion at pH 7.0 was assumed to be 1.0 (100%). If the observed pH minimum for a species in the field is 6.0, the species is considered extinct at 5.9. The curves for each group followed a sigmoid trend with declining pH. Joseph Eilers (pers. comm., Northrop Services, Corvallis, Oregon) provided us with the original database allowing us to reconstruct the pH-minima curves rather than interpolate from a graph in the published report. The data on sponges were not used here because of the small number of species.

Mills and Schindler (1986) brought the curves of Eilers et al. (1984) to a wider audience. They showed that the compiled curves mirrored the biotic response to declining pH at the ELA Lake 223 acidification experiment. Subsequently, Schindler et al. (1989) developed a model of biotic damage based on the curves of Eilers et al. (1984). The pH-minima curves are used to estimate the percentage loss of potential richness when a lake's pH is changed from an original, nonacidified, level to that resulting from the input of acidic deposition as shown in Fig. 2a where the curve is the mean of the sigmoid curves for the seven taxonomic groups considered. Schindler et al. (1989) divided the proportional loss of potential richness $(P_0 - P_a)$ by the original proportion (P_0) to estimate the relative damage (Fig. 2b). A 20% loss applied at various points along the curve (Fig. 2a) results in a rising relative value with declining pH thereby exaggerating damage in lakes which had a low pH originally and producing a measure of damage which is not comparable at different points along the pH axis. Here we have used the actual percentage loss of potential richness $(P_0 - P_a)$ as a measure of biotic damage. This latter value is directly proportional to numbers of species if the actual original richness is known.

We have used the phrase 'potential richness' to highlight the fact that we are not predicting changes in actual richness in individual lakes. The assemblage of species found in a partic-

TABLE 1. Predicted mean percentage loss of richness for seven groups of aquatic biota and the mean percentage loss of the seven groups using the 1980 deposition scenario by secondary watershed and region in eastern Canada. Highest and lowest regional means with asterisk and underline, respectively.

Region	Algae	Rotifers	Leeches	Molluscs	Crustaceans	Insects	Fish	Groups' mean
Ontario mean	5.9	4.3	5.3	6.2	4.2	5.6	6.6*	5.4
2A	0.1	0.0	0.3	0.2	0.1	0.2	0.0	0.2
2B	1.9	0.9	1.9	2.2	1.3	2.1	1.8	1.7
2C	22.6	17.5	18.8	22.0	16.3	20.3	25.8	20.5
2D	25.4	18.3	22.4	25.7	17.7	23.5	28.0	23.0
2E	27.7	20.2	23.7	27.5	19.3	25.2	30.5	24.9
2H	17.5	15.9	13.7	16.6	14.7	15.3	21.8	16.5
2J	9.8	4.6	10.0	10.1	5.6	9.9	8.2	8.3
2K	18.2	13.8	13.7	16.7	12.8	15.7	20.8	16.0
4G	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4J	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4L	0.4	0.4	0.6	0.7	0.4	0.6	0.5	0.5
4M	0.4	0.4	0.6	0.7	0.4	0.6	0.5	0.5
4N	0.3	0.1	0.5	0.5	0.3	0.4	0.3	0.3
5P	0.8	0.4	0.6	0.6	0.4	0.7	0.6	0.6
5Q	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5R	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Quebec mean	11.0	5.7	10.6	12.4*	6.4	11.1	10.7	9.7
2J	9.3	1.6	9.8	8.0	3.7	9.0	4.5	6.6
2K	12.8	4.7	7.8	8.8	5.2	10.0	10.5	8.5
2L	7.7	4.0	8.4	8.4	5.0	8.0	6.5	6.9
2N	17.7	6.8	16.3	16.8	8.5	17.1	13.2	13.8
2O	4.8	1.0	6.3	5.2	2.5	5.4	2.2	3.9
2P	14.7	5.8	12.9	14.0	6.7	13.6	12.0	11.4
2R	8.4	3.4	9.0	9.9	4.8	8.9	6.8	7.3
2S	14.4	12.3	14.7	18.2	11.5	14.9	19.0	15.0
2T	10.5	7.3	9.8	12.7	7.0	10.8	11.5	9.9
2U	11.5	8.1	10.8	14.0	7.9	11.8	12.9	11.0
2V	12.9	6.2	11.0	14.8	6.7	12.4	13.4	11.1
2W	11.4	5.2	9.9	12.9	6.0	10.9	11.6	9.7
2X	10.5	4.6	8.8	11.6	5.4	9.9	10.6	8.8
3A	10.6	5.5	11.0	12.2	6.5	11.1	9.6	9.5
3B	7.4	2.6	8.0	8.1	4.0	7.8	5.4	6.2
Maritimes mean	8.6*	4.6	7.4	8.6*	4.6	8.2	7.6	7.1
1A	21.7	17.2	21.3	24.5	16.2	21.5	25.1	21.1
1B	16.1	10.8	15.5	19.5	10.5	16.7	17.4	15.2
1D	18.8	12.2	14.6	22.0	11.3	18.0	21.8	17.0
1E	17.7	21.6	17.5	26.9	14.6	19.6	29.3	21.0
1F	15.2	9.0	12.3	17.8	8.6	14.8	17.0	13.5
2Y	6.0	3.7	6.0	6.7	3.6	6.0	6.0	5.4
2Z	10.2	3.7	7.6	8.3	4.5	9.0	7.2	7.2
E. Canada mean	8.9	5.0	8.3	9.5	5.3	8.6	8.5	7.7

ular lake is a subset of the pool of species present in the region and actual richness may be determined by other factors such as lake size (Matuszek and Beggs 1988; Minns 1990).

We predicted biotic damage using predicted cumulative distributions of pH in populations of lakes by watershed and emission–deposition scenarios in combination with the potential richness model. The distribution of original pH values was used to derive P_0 values at each percentile point from 1 to 100 with an interval of 1. Then for each scenario, the same procedure was used to derive P_a values. Percentiles from both the original and scenario distributions were paired and subtracted to give the predicted percentage loss of richness ($P_0 - P_a$) distribution by percentiles. The percentile distributions of percentage loss were summarised in three ways: (1) tabulation of the mean percent loss of potential richness ($P_0 - P_a$) by secondary watershed, (2) plots of cumulative percentiles of lakes ordered by decreasing values of ($P_0 - P_a$), and (3) shaded regional maps showing those secondary watersheds meeting dual criteria of ($P_0 - P_a$) values and cumulative percentage of lakes affected.

Results

Given the predicted percentile distributions of pH for lakes by secondary watershed under the 1980-base deposition scenario and prior to the occurrence of acidic deposition (Jones et al. 1990), the mean percentage richness loss ($P_0 - P_a$) ranged from 0.0 to 30.5 across the seven taxonomic groups of aquatic biota and 38 watershed areas (Table 1). The seven groups showed a similar response pattern across watersheds. Rotifers and crustaceans were predicted to have the lowest losses and molluscs, algae, and fish the highest losses with the other two groups (leeches and insects) being closer to the highest values.

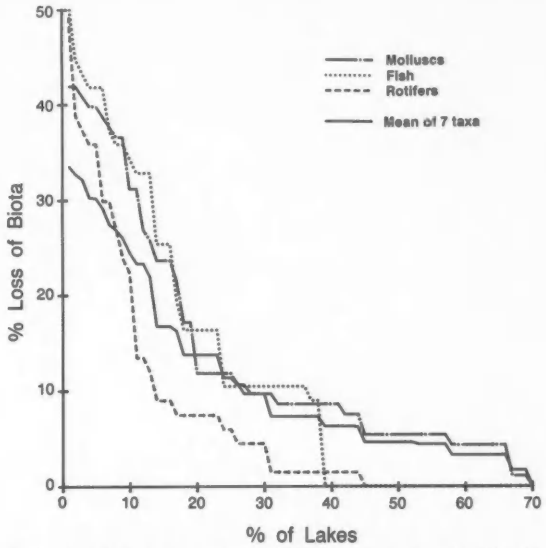

FIG. 3. Cumulative percentage of lakes in eastern Canada (X axis) predicted to have a given or greater percentage loss of richness (Y axis) given the 1980 deposition scenario: Molluscs, fish, rotifers, and the mean of seven taxonomic groups.

Molluscs were predicted to have the highest mean losses among groups in 16 of 38 watersheds and fish in eight of 38. Within watersheds, the unweighted mean loss was representative of the range, e.g. 24.9 versus 19.3 to 30.5% in Ontario watershed 2E and 15.0 versus 11.5 to 19.0% in Quebec watershed 2S. Therefore the unweighted groups' mean percent loss of richness for the seven groups was considered to be a good indicator of the losses for each watershed.

Across eastern Canada, the groups' mean losses showed considerable variation as was expected from the variations in the distribution of lake chemical sensitivity and in the level of acidic deposition. In Ontario, watersheds with the highest group means were clustered in the south-central portion of the province on the Canadian Shield, e.g. watersheds 2C, 2D, 2E, 2H, and 2K had mean losses of 16.0 to 24.9%. In Quebec, the range of losses was less than in Ontario with the highest values occurring in watersheds 2P (groups' mean 11.4%), 2N (13.8), and 2S (15.0). Groups' mean values were higher in the Maritimes than in Quebec with the highest mean losses being in watersheds 1A (21.1%) in New Brunswick and 1E (21.0) in Nova Scotia. Over all of eastern Canada south of latitude 52, the predicted groups' mean potential loss of aquatic biota was 7.7 with a range of mean losses among the seven taxonomic groups of 5.0 to 9.5%.

While the mean response may be a good indicator, the distribution of responses within a watershed may be uneven with some lakes sustaining more damage and others less. Within a region, lakes with high original alkalinities ($> 150\ \mu eq \cdot L^{-1}$) are relatively unaffected while lakes with low ones ($< 50\ \mu eq \cdot L^{-1}$) are altered considerably. To examine this situation, we ordered the percentiles of lakes by their percentage loss of richness and plotted the cumulative percentage of lakes having a given or greater level of damage to show the distribution of damage under the various deposition scenarios. While the groups' mean loss was predicted to be 7.7% for all of eastern Canada, losses greater than 30% were predicted in about 5% of lakes (Fig. 3). Rotifers were predicted to be the least affected taxonomic group and molluscs and fish the most affected with losses of more than 10% in more than 25% of lakes. Although the pH minima curve for molluscs begins to decline at higher pH's than does the fish curve (Eilers et al. 1984), there are relatively few high pH lakes (Jones et al. 1990). Therefore when the chemical status distributions were linked to the biological response curves of the potential richness model, the damage distributions showed fish and molluscs were affected to similar levels in more than 35% of lakes but fish were unaffected in more than 60% of lakes. The mean curve for the seven groups of biota fell below the mollusc curve for the first 20% of lakes and followed the mollusc curve thereafter.

While the cumulative damage curves illustrate the distribution of damage among populations of lakes in a region, they cannot be used to illustrate the spatial pattern of biotic damage. However, these results did suggest that dual criteria incorporating two percentage values might be used to prepare a statement of biotic damage: the percentage of lakes in each area exhibiting a given or greater percentage loss of potential richness.

We examined a range of dual percentage criteria, from 10 to 50%, to find a combination which highlighted the contrast between the various deposition scenarios and between taxonomic groups. We arbitrarily selected 20:20, i.e. 20% or more of lakes predicted to have 20% or more loss of potential richness, on the grounds that 20% is a significant portion of any resource. Taking the 1980 deposition scenario, we looked at the geographical distribution of secondary watersheds meeting the criteria for fish, molluscs, rotifers, and the mean of seven taxonomic groups (Fig. 4). The map for molluscs (Fig. 4B) showed a large proportion (53%) of watersheds met the 20:20 criteria, whereas the map for rotifers (Fig. 4D) showed a substantially smaller proportion (26%). The groups' mean and fish maps showed similar but intermediate extents of effect. Lake resources in large portions of eastern Canada were predicted to have 20:20 damage given levels of acidic deposition prevailing in 1980. As indicated (Table 1), the affected areas were mainly south-central Ontario, Quebec along the north shore of the St. Lawrence, and the southern Maritimes (Fig. 4).

Using the groups' mean percent loss of richness, we examined the mean losses predicted as a result of the three emission control scenarios relative to the 1980 deposition scenario and the current conditions (Table 2). For the 42% reduction in Canadian emissions, the biggest recoveries were predicted for south-central Ontario and the smallest for the Maritimes. When emissions were reduced by 42% in both Canada and the United States, there were further recoveries in all three regions, especially in Ontario. A 10% increase in emissions caused the mean loss of richness in affected watersheds to increase slightly (1–2%). The estimated current mean loss of richness based on the regional lake survey data was the lowest of all scenarios for most watersheds. Watersheds in Ontario around Sudbury (2C and 2D) and in Nova Scotia (1D, 1E, 1F) had current values similar to or worse than those predicted for 1980 emission levels. Most watersheds were predicted to sustain further lake acidification and therefore further biotic losses as they came into a steady state with 1980 emission levels. According to the lake survey data (Kelso et al. 1986), current lake sulphate concentrations were lower than values predicted from sulphate deposition in 1980 and therefore were not in steady state with deposition chemistry. Therefore predicted lake conditions under

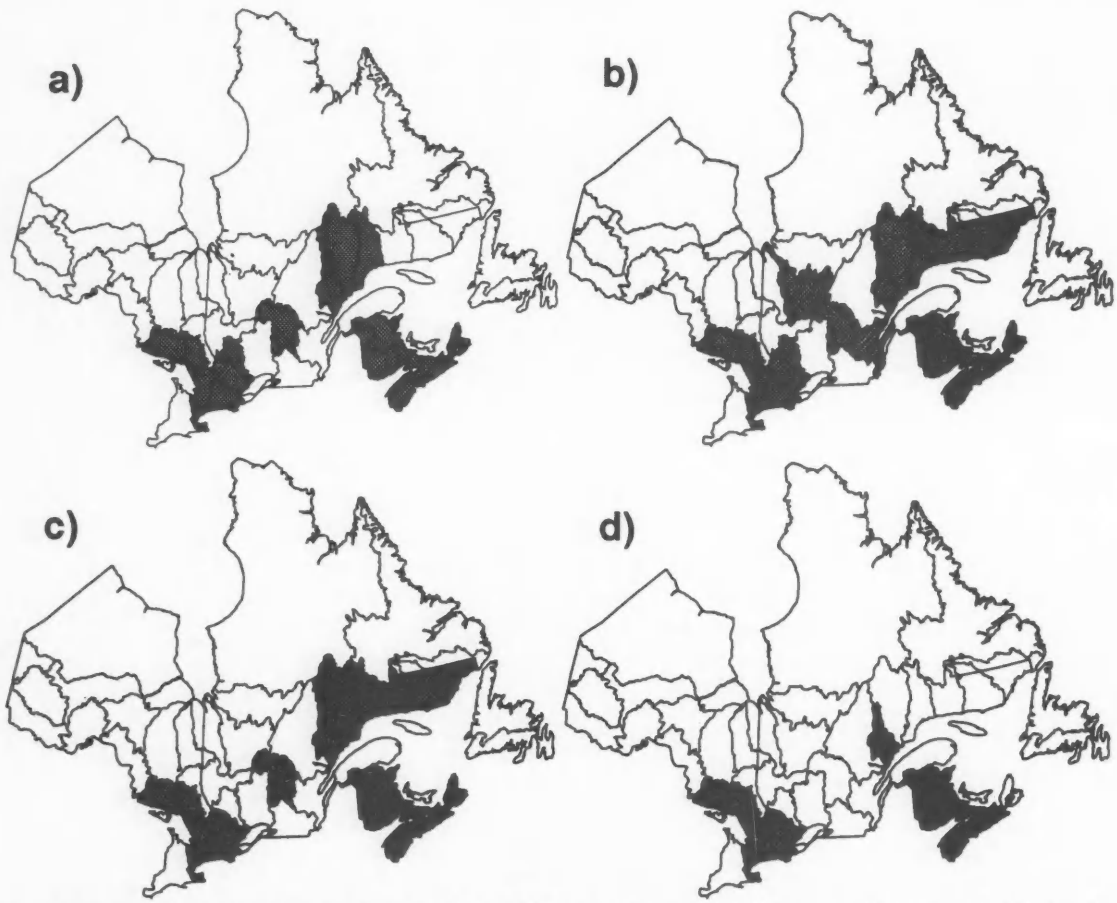

FIG. 4. Secondary watersheds in eastern Canada predicted to have 20% or more richness loss in 20% or more of lakes given the 1980 deposition scenario: (a) the mean of seven taxonomic groups, (b) molluscs, (c) fish, and (d) rotifers.

different emission control scenarios must be assessed against the predicted steady state conditions under the levels of acidic deposition prevailing in 1980 and not against the current conditions measured in the surveys.

The percentile distributions of damage showed the response to the emission control scenarios was largely confined to about 30% of the lakes with the most affected portions of the lake populations showing the greatest improvements (Fig. 5). None of the emission reductions reduced the level of percentage loss below 25% for all portions of the lake population; varying percentages of the lake populations exhibited levels of loss greater than 20%.

When the dual criteria were applied, the patterns of watersheds predicted to have 20% or more mean losses in 20% or more of lakes varied considerably under the various deposition scenarios (Fig. 6). The current chemical conditions predicted that those damage levels were present in a few south-central Ontario watersheds and throughout New Brunswick and Nova Scotia. The pattern expanded to include more watersheds in Ontario and a large part of Quebec under the 1980 deposition scenario. The Canadian emission controls were predicted to reduce the affected areas by three watersheds in Quebec and by one each in Ontario and the Maritimes. Adding United States controls removed the remaining effects from Nova Scotia (1D, 1E) and one watershed in Ontario. Increased emissions expanded the area affected, particularly in Quebec.

Discussion

Uncertainties in the Chemical Predictions

The predictions of the site model have been shown to be most sensitive to uncertainty in the values of acidic sulphate deposition and original sulphate (M. L. Jones, K. J. Heltcher, D. R. Marmorek, and C. K. Minns, unpubl. data). Deposition monitoring networks in Canada are few in number and deposition rates for most areas are obtained by interpolation. There is also evidence of considerable variation at individual sites depending on the placement of collectors. There are no data sets describing either the original sulphate concentrations in lakes prior to the occurrence of acidic deposition or original sulphate deposition from the atmosphere plus any inputs from soils in the watersheds. Yet, it is essential that we be able to estimate the change in lake sulphate or the change in sulphate

TABLE 2. Predicted mean percentage loss of richness for the mean of seven groups of aquatic biota given five emission scenarios by secondary watershed and region in eastern Canada.

Region	1980	42% Decrease		10% Increase	Current
		Canada	Canada + United States		
Ontario mean	5.4	4.5	3.4	6.0	4.2
2A	0.2	0.2	0.1	0.2	0.2
2B	1.7	1.5	1.0	1.8	1.5
2C	20.5	18.3	14.2	21.8	21.8
2D	23.0	16.9	12.0	25.2	23.1
2E	24.9	17.6	12.3	27.1	10.6
2H	16.5	13.8	11.4	17.4	4.1
2J	8.3	5.8	4.0	9.3	7.1
2K	16.0	12.5	9.5	17.1	3.5
4G	0.0	0.0	0.0	0.0	0.0
4J	0.0	0.0	0.0	0.0	0.0
4L	0.5	0.5	0.4	0.6	0.1
4M	0.5	0.5	0.3	0.6	0.1
4N	0.3	0.3	0.1	0.4	0.1
5P	0.6	0.6	0.5	0.6	0.2
5Q	0.0	0.0	0.0	0.0	0.0
5R	0.0	0.0	0.0	0.0	0.0
Quebec mean	9.7	7.8	6.0	10.6	7.4
2J	6.6	4.2	3.0	7.3	6.9
2K	8.5	4.6	2.4	9.9	6.0
2L	6.9	5.4	4.2	7.4	4.5
2N	13.8	10.5	7.8	15.1	8.2
2O	3.9	3.1	2.2	4.5	4.2
2P	11.4	9.3	7.1	12.3	10.6
2R	7.3	5.9	4.7	8.1	7.1
2S	15.0	12.7	10.7	16.0	8.9
2T	9.9	8.0	6.2	11.0	9.5
2U	11.0	8.9	7.2	11.6	9.5
2V	11.1	9.0	6.7	12.2	6.5
2W	9.7	8.1	6.6	10.7	6.5
2X	8.8	7.3	6.0	9.5	6.3
3A	9.5	7.2	5.3	10.4	6.8
3B	6.2	5.1	4.3	6.6	7.2
Maritimes mean	7.1	6.4	5.5	7.6	6.6
1A	21.1	19.4	16.0	22.1	14.7
1B	15.2	13.4	10.4	16.3	15.0
1D	17.0	14.5	9.9	19.0	24.2
1E	21.0	18.0	12.5	23.0	36.8
1F	13.5	12.1	10.4	14.5	24.2
2Y	5.4	4.9	4.3	5.8	2.6
2Z	7.2	6.5	5.6	7.5	9.8
E. Canada mean	7.7	6.5	5.2	8.4	6.3

deposition if we are to predict the degree of acidification. Other factors, like F_w and runoff, affect the lake chemistry predictions, but to a lesser degree. Taken alone F_w, the proportion of neutralization in the watershed, is important but not in the aggregate if one accepts that F_w varies monotonically in some fashion with original cation levels which ought to reflect background cation release rates from the rocks and soils of the watershed.

Our ability to predict the chemistry of lakes in relation to varying levels of acidic sulphate deposition hinges on a number of assumptions. Many of those assumptions are not readily tested but represent the prevailing consensus and err in a conservative manner, e.g. F_w varies with our estimates of original base cation levels but reaches a maximum value of 1

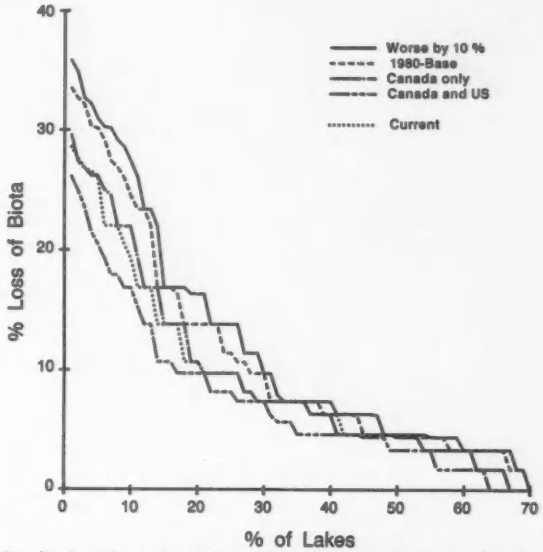

FIG. 5. Cumulative percentage of lakes in eastern Canada predicted to have a given or greater percentage richness loss for the mean of seven groups of aquatic biota given: a 10% increase of emissions, 1980 deposition, a 42% reduction of Canadian emissions, a 42% reduction of Canadian and United States emissions, and current conditions.

at a relatively low level of base cations. At the very least, the predictions may be considered useful on a relative scale. This view is probably too conservative, since the disagreements between values predicted from 1980 deposition levels and the 'current' condition as measured in surveys were not substantial (Jones et al. 1990).

In connection with our evaluation of emission control scenarios relative to predicted 1980 and observed 1979 to 1982 lake chemistry in eastern Canada, we must note that decreases in deposition and improvements in lake chemistry have been observed in the Sudbury area of Ontario between 1974 to 1976 and 1981 to 1983 (Keller and Pitblado 1986) and in the Algoma district in Ontario between 1979 and 1985 (Kelso and Jeffries 1988). Kelso and Jeffries (1988) also found evidence of reinvasion and expansion of fish stocks in some lakes where pH had increased. Thus the situation now, in 1990, likely lies somewhere between the conditions predicted in 1980 and those predicted with a 42% emission reduction in Canada and the United States.

Percent Loss of Species Richness

The model of biotic damage used here could overstate the amount of loss because potential compensatory mechanisms are ignored. If, as is proposed in the theory of island biogeography (MacArthur and Wilson 1967), the species richness in a lake, another sort of island, is a result of the balancing of immigration and extinction rates, lake acidification will increase the extinction rate and thereby reduce richness. There may be a delay in the establishment of a new richness equilibrium due to low rates of immigration and/or an associated lag in the increase of the immigration rate. Observed species richness may fall below the equilibrium point. After the initial rise, extinction rates will gradually decline to an intermediate level with the arrival of acid-tolerant species and the lessened extinction rates for the

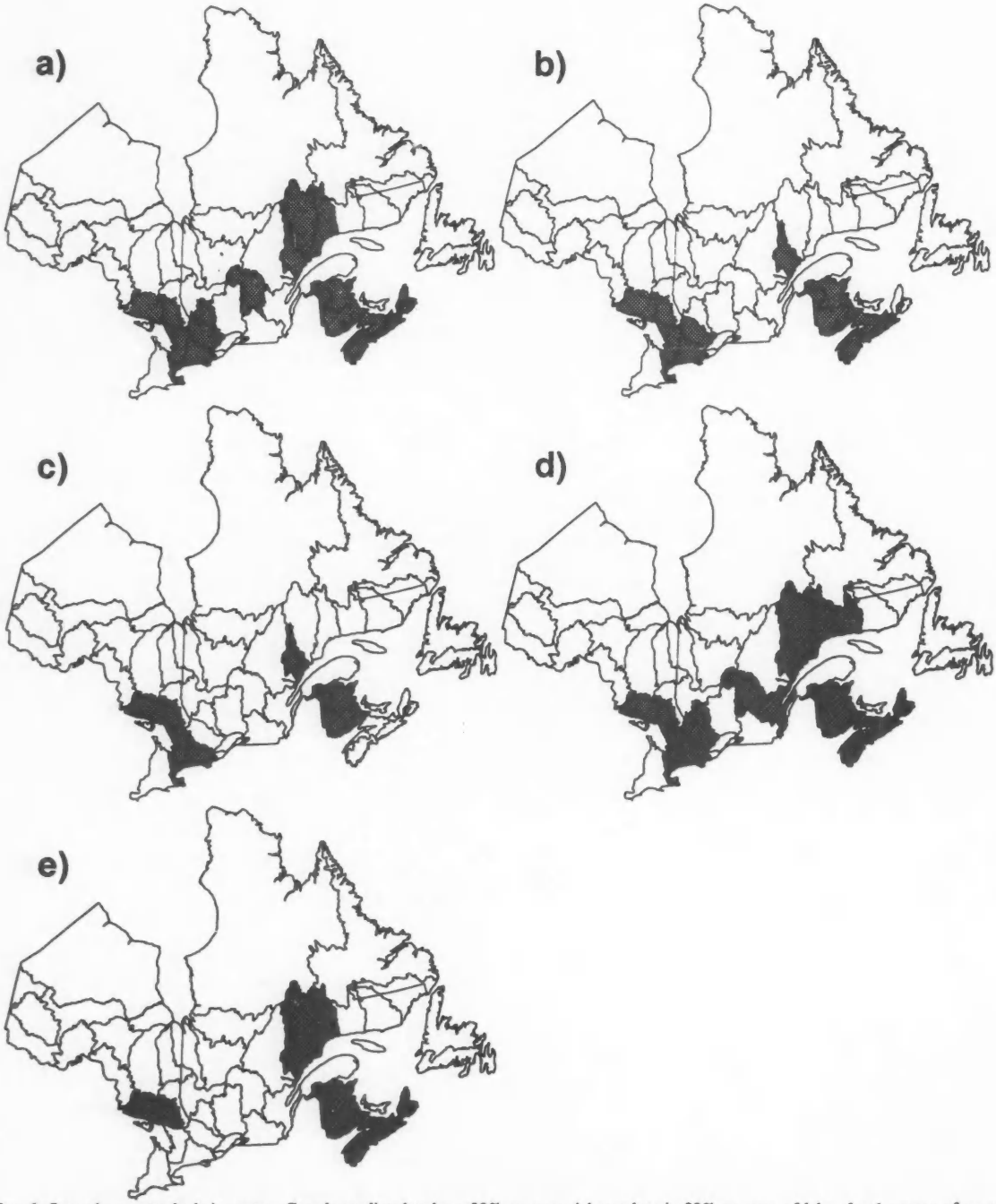

FIG. 6. Secondary watersheds in eastern Canada predicted to have 20% or more richness loss in 20% or more of lakes for the mean of seven groups of aquatic biota given: (a) 1980 deposition, (b) a 42% reduction of Canadian emissions, (c) a 42% reduction of Canadian and United States emissions, (d) a 10% increase of emissions, and (e) current conditions.

species persisting in the lake. However, if acidification is a regional problem affecting large numbers of lakes, as is predicted for eastern Canada, immigration rates might also be reduced through a widespread reduction in species richness that limits the supply of immigrants to acid-tolerant species, thereby decreasing compensation. This would be true if the size of the

regional species pool influences the level of immigration. Southwood and Kennedy (1983) distinguished between immigrants and colonizers; the former include many species which enter new ecosystems but fail to persist and the latter include those which arrive and succeed. Among the immigrants will be acid-intolerant species. Extinction only applies to those species 'established' in the ecosystem. Where lake acidification occurs, there may be many immigrants but few colonizers. Thus it is likely the percentage loss of biotic richness may overestimate the level of damage but unlikely immigration can completely replace acid-related losses.

The percent loss of richness model can be used to describe both loss and recovery but only at steady state. Long-lived species may linger in a lake long after reproduction has ceased. As noted above, recovery may be long-delayed by low immigration rates particularly in larger forms such as fish which have fewer opportunities to move between lakes and take longer to mature and produce a population compared to smaller organisms like insects and algae.

Among the disadvantages of the biotic damage model used here are the equal treatment of all species within a taxonomic group and the inability to reflect the differential values humans attach to different species. Within taxonomic groups a lake's biomass and production are unevenly allocated among the species present and the acid-sensitive species may account for a large portion of the total. Humans selectively exploit piscivores such as lake trout (*Salvelinus namaycush*) and smallmouth bass (*Micropterus dolomieui*) and loss of populations of these and similar species is considered to be of far greater importance than the loss of less valued species.

The species richness losses considered here and in Eilers et al.'s (1984) compilation are more closely linked to regional species pools than to the richness of individual lakes. Minns (1990) has shown that local (i.e. lake) richness is usually a small proportion of regional (i.e. secondary watershed) richness. In keeping with island biogeography concepts (MacArthur and Wilson 1967), local richness is related to lake size and associated habitat constraints. Regional richness is tied to evolutionary and geological events (Ricklefs 1987). A next step in the development of richness models as a basis for measuring the impact of acidification and other stresses should be the development of local richness models. Lake richness models tied to the potential richness model should provide a basis of making absolute estimates of the numbers of species populations lost or recovered in relation to changing emission levels.

Predicted Scale and Extent of Biotic Damage

We have chosen relatively high damage criteria as a basis for damage assessment: 20% or greater percentage loss of richness in 20% or greater of lakes in a watershed or region. Dual criteria are needed to allow consideration of both the level of response and the absolute magnitude and extent of effects. In percentage terms, the predictions shown here are little different from the chemical predictions reported earlier (Jones et al. 1990). However, measuring the damage in biological units directly addresses the primary basis for our concern about the potential impacts of acidic deposition. Schindler et al. (1989) predicted comparable levels of biotic damage in lakes across the northeastern United States. The predictions shown here indicate there was already a large area showing the effects of lake acidification and that if 1980 acidic deposition levels had prevailed, more losses would have occurred. The emission reduction scenarios predict that the expected level of damage can be reduced. Indeed in Ontario, Kelso and Jeffries (1988); Keller and Pitblado (1986); and Beggs and Gunn (1986) have already reported improvements in lake chemistry and biota in Algoma and Sudbury area lakes in response to decreases in acid deposition. However, the scenarios presented here also indicate further emission reductions beyond those already achieved, being implemented, or proposed will be needed if biotic losses are to be reduced to a minimum.

Acknowledgements

We wish to note the work of our colleagues, Dave Marmorek and Floyd Elder, on the integrated regional model of acidification. We thank John Kelso and Ken Mills in Sault Ste. Marie and Winnipeg, respectively for comments and suggestions on an earlier draft. The comments of the reviewers (Mark Hansen and Anonymous) helped polish the final draft.

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Effects of Temperature on Metabolism of Benzo[*a*]pyrene by Toadfish (*Opsanus beta*) Hepatocytes

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Gill, K. A., and P. J. Walsh. 1990. Effects of temperature on metabolism of benzo[a]pyrene by toadfish (*Opsanus beta*) hepatocytes. Can. J. Fish. Aquat. Sci. 47: 831–837.

Prior in vivo studies of the effects of temperature on uptake, metabolism and excretion of benzo[a]pyrene (BaP) by the gulf toadfish (*Opsanus beta*) suggested that metabolism of BaP may be partially temperature insensitive under certain conditions. In the present study, hepatocytes were isolated from toadfish acclimated to 18 or 28°C, and then cells were exposed to BaP at 18, 23, and 28°C in vitro to more directly examine the effects of temperature on metabolism. Toadfish hepatocytes metabolized BaP to a variety of Phase I and Phase II metabolites in similar proportions to toadfish in vivo, with the exception that fewer Phase I metabolites were detected. A marked temperature dependence of metabolism of BaP to Phase II metabolites was noted between 18 and 28°C at near saturating concentration of BaP (13 $\mu g \cdot mL^{-1}$). Warm-acclimated toadfish displayed a lower temperature sensitivity than cold-acclimated toadfish (Q_{10}, the ratio of the rate at $T + 10°C$: rate at $T = 1.85$ and 2.65, respectively). However, at a lower, subsaturating concentration of BaP (5 $\mu g \cdot mL^{-1}$), production of all metabolites showed a marked temperature independence. We speculate that this independence is the result of temperature effects on the kinetic properties of the enzymes of xenobiotic metabolism.

Nos études in vivo précédentes sur les effets de la température sur l'incorporation, le métabolisme et l'excrétion du benzo[a]pyrène (BaP) par un poisson-crapaud (*Opsanus beta*) laissant entendre que le métabolisme du BaP pourrait être partiellement insensible à la température sous certaines conditions. Dans la présente étude, des hépatocytes provenant de poissons-crapauds acclimatés à des températures de 18 ou 28°C ont été isolés puis mis en présence de BaP à 18, 23 et 28°C in vitro pour étudier directement les effets de la température sur le métabolisme. Le BaP a été métabolisé par les hépatocytes et a été transformé en différents métabolites de phase I et de phase II dans des proportions similaires à ce que nous avons observé chez les poissons-crapauds in vivo. Cependant, nous avons détecté peu de métabolites de phase I. Nous avons observé que la température avait un effet marqué sur la production de métabolites de phase II entre 18 et 28°C quand la concentration de BaP s'approchait du point de saturation (13 $\mu g \cdot mL^{-1}$). La sensibilité à la température était moins grande chez les poissons-crapauds acclimatés à une température de 28°C que chez les poissons-crapauds acclimatés à 18°C (Q_{10}, rapport du taux à $T + 10°C$: taux à $T = 1,85$ et 2,65, respectivement). Cependant, quand la concentration de BaP était plus faible et nettement en-dessous du point de saturation (5 $\mu g \cdot mL^{-1}$), la production de tous les métabolites était tout à fait indépendante de la température. Nous pensons que cette indépendance serait attribuable aux effets de la température sur les propriétés cinétiques des enzymes du métabolisme xénobiotique.

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Recently, an interest has developed in the use of ectothermic vertebrates, particularly fishes, in the study of the mechanisms of carcinogenesis and in carcinogenicity testing (Hoover 1984; Powers 1989). Since the body temperature of most fishes is variable, if fish are to serve as adequate model systems for the study of carcinogenesis, the effects of temperature on carcinogenesis must be known (Hoover 1984). In the few studies of temperature on carcinogenesis in fish, temperature has been implicated as a modulating agent. In the European eel, *Anguilla anguilla*, fewer papillomas were observed in winter fish, and in some cases tumor regression was observed when summer fish were transferred to colder temperatures in the laboratory (Peters and Peters 1977). Further, the medaka, *Oryzias latipes*, developed fewer liver tumors when exposed to diethylnitrosamine at cold temperatures versus exposure at warm temperatures (Kyono-Hamaguchi 1984). Recently, we have begun to examine possible mechanisms of the effects of temperature on chemical carcinogenesis, and have found that gulf toadfish (*Opsanus beta*), when exposed at colder temperatures, take up less of the carcinogen benzo[*a*]pyrene (BaP) (Kennedy et al. 1989a) and produce fewer of the Phase I metabolites (i.e., initial oxidation products of monooxygenases) associated with enhanced carcinogenesis (i.e., tetrol and triol breakdown products of diol epoxides) (Kennedy et al. 1989b). In addition we were able to infer that overall rates of metabolic processing of BaP to Phase II metabolites (e.g., end products as sulfate, glucuronide, and glutathione conjugates of Phase I metabolites with enhanced aqueous solubility) by the liver may be partially independent of temperature; our interpretation, however, was complicated because the observed in vivo effects may have resulted from thermal alterations of uptake rates (Kennedy et al. 1989a,b).

Suspensions of isolated fish hepatocytes are an established experimental tool in studies of metabolic biochemistry in fish

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(Moon et al. 1985), including toadfish (Walsh 1987; Mommsen and Walsh 1989), and in particular in studies of xenobiotic (foreign substance) metabolism (e.g., Parker et al. 1981; Bailey et al. 1984; Morrison et al. 1985; Andersson 1986). Andersson and Koivusaari (1986) have demonstrated that metabolism of xenobiotics by rainbow trout hepatocytes is influenced by acclimation temperature and exposure temperature, indicating that hepatocytes may be a good experimental system for further studies of the effects of temperature on BaP metabolism in *Opsanus beta*.

The purpose of this study was to (1) examine the ability of hepatocytes from a marine teleost, *Opsanus beta*, the gulf toadfish, to metabolize the carcinogen BaP to Phase I and II metabolites; and (2) examine the effects of acute and acclimatory temperatures on BaP metabolism by toadfish hepatocytes to test the hypothesis of temperature independence suggested by our in vivo observations.

Materials and Methods

Experimental Organism and Acclimation Regimen

Specimens of the gulf toadfish (*Opsanus beta*), weighing 37 to 421 g, were collected from Biscayne Bay, Florida by roller trawl by a local fisherman in May and June 1988. The fish were separated into two groups and acclimated for at least 4 wk in flow-through seawater aquaria to water temperatures of 18 or 28°C ± 1.0°C and a photoperiod of 12 h light:12 h dark. These temperatures reflect typical winter and summer water temperatures in Biscayne Bay (Sogard et al. 1987). Fish were fed chopped frozen squid ad libitum until 3 d before an experiment. Fish were sexed during the hepatocyte isolation.

Chemicals

[7,10-^{14}C] BaP (50.5 mCi·mmol^{-1}) was purchased from Amersham (Arlington Hts., IL). The radiochemical purity of [^{14}C] BaP as determined by thin-layer chromatography and high-performance liquid chromatography (HPLC) exceeded 99%. BaP metabolite standards were obtained from the National Cancer Institute Chemical Repositories, Midwest Research Institute (Kansas City, MO) and unlabelled BaP was obtained from Kodak (Rochester, NY). All other chemicals (reagent grade) and biochemicals were purchased from Sigma Chemical (St. Louis, MO).

Isolation of Hepatocytes

Hepatocytes were isolated from toadfish as in Walsh (1987) by a modification of procedures developed initially for rainbow trout (French et al. 1981; Walsh 1986). Briefly, livers were perfused initially with a Ca^{2+} free Hanks salts solution for 5 min, and then collagenase (95 units·mL^{-1}, Sigma C5138) and hyaluronidase (120 units·mL^{-1}, H3506) were added to the perfusate and perfusion was continued for 25 min. The hepatocytes were then gently disaggregated by filtration through nylon mesh and concentrated by low-speed centrifugation. Purified hepatocytes were suspended in a Hanks salts solution to which was added 10 mM HEPES, 1 mM $CaCl_2$, 4% bovine serum albumin (Sigma A4503), 3 mM glucose and 1 mM lactate. The medium was equilibrated with 0.25% CO_2, balance air, and the final pH was adjusted to 7.70 (Walsh 1987). Viability was determined as the percent of cells excluding trypan blue (Walsh 1987).

Exposure of Hepatocytes to [^{14}C] Benzo[*a*] pyrene and Experimental Design

Unlabelled BaP in acetone or [^{14}C]-BaP in toluene were dried under a stream of nitrogen and then dissolved in dimethyl sulfoxide (DMSO). This stock solution was added to suspension medium, and 0.9 mL of this medium was dispensed to 7 mL amber vials. To initiate an exposure, 0.1 mL of cells in suspension medium was added to each vial and the vials were immediately transferred to temperature baths at 18, 23, or 28°C. Final volume of DMSO was 1.6 μL·mL^{-1} (i.e., 22.6 mM). An aliquot of the suspension medium was reserved for liquid scintillation counting and HPLC to measure the initial BaP concentration. An aliquot of the cell stock was centrifuged for 1 min at 13 000× g to determine cell wet weight (typically 25 to 30 mg or 3 × 10^6 cells, per millilitre suspension medium final concentration). After 1.5 h, reactions were terminated by the addition of 1 mL of methylene chloride to each vial followed by vigorous shaking. Duplicate determinations were run for each temperature for each fish. Two types of controls were performed routinely: addition of methylene chloride to the medium before addition of cells, and incubation of suspension medium and BaP without cells (methylene chloride added after 1.5 h). This procedure was repeated for all fish. In one experiment we also tested two other control procedures, namely acidifying cell suspensions with 0.1 mL 70% perchloric acid and brief boiling of cell suspensions, prior to addition to suspension and BaP.

Extraction and Analysis of BaP and BaP-metabolites

The methylene chloride-media mixtures were transferred to conical glass centrifuge tubes and the amber vials were rinsed with an additional 1 mL of methylene chloride and transferred to the centifuge tubes. The organic layer was separated from the aqueous layer by centrifugation, and the aqueous layer was further extracted with 2 mL of methylene chloride. A 100-μL aliquot was removed from the organic fraction for scintillation counting. The remaining organic fraction was evaporated under nitrogen and redissolved in 100 μL methanol and stored at −80°C until 30 μL was used for HPLC analysis of BaP and Phase I metabolites (i.e., total organic soluble fraction). HPLC analysis was by the methods of Elnenaey and Schoor (1981) as previously applied to toadfish bile (Kennedy et al. 1989a). The method employs both a fluorescence detector and a fraction collector to identify (by comparison of elution times to those of standards) and to quantify Phase I metabolites of BaP. When comparisons of elution times were ambiguous (e.g., especially with the BaP diones) samples were reanalyzed with co-injected standards. In preliminary experiments, aliquots of several BaP Phase I metabolite standards (including the less hydrophobic tetrols and triols) were added to samples of toadfish bile and extracted as above. Percent recovery was in excess of 90% for all standards tested as in Kennedy et al. (1989b).

A 100-μL aliquot was removed from the remaining aqueous layer for scintillation counting to quantify the total Phase II metabolites (i.e., total aqueous soluble fraction) produced. To quantify glucuronide and sulfate conjugates we used the methods previously developed for English sole (Varanasi et al. 1983). The remaining aqueous layer was first incubated for 24 h at 37°C with 15 units of β-glucuronidase (Sigma, 105-2000B, free of sulfatase activity). The solution was then extracted three times with methylene chloride. The organic extracts were combined, concentrated by evaporation, and an aliquot reserved for scintillation counting to quantify glucuronide conjugates. A 30-

μL aliquot was used to HPLC analysis to determine which Phase I metabolites were being conjugated to glucuronides. Efficiency of glucuronidase hydrolysis and extraction of a 9-Benzo[*a*]pyrene-*B*-*D*-glucopyranosiduronic acid standard was >86% of acid hydrolysis at 80°C and extraction of the same standard.

The aqueous layer remaining after hydrolysis and extraction of glucuronide conjugates was incubated for 24 h at 37°C with 0.5 units of sulfatase (Sigma, S 1629, free of glucuronidase activity). The aqueous layer was again extracted three times with methylene chloride, the organic layers were combined, concentrated, and subjected to scintillation counting and HPLC as above to determine the total sulfates and which Phase I metabolites were conjugated to sulfates. An aliquot of the remaining aqueous layer was counted for radioactivity (usually a substantial component of the total) to quantify the remaining Phase II metabolites, presumably glutathione conjugates (Varanasi et al. 1983; Stein et al. 1987).

Aliquots from the various extractions, or fractions from the HPLC analysis, were counted for total ^{14}C activity on a Tracor Analytic Beta Trac Model 6895 liquid scintillation counter. Aqueous samples were combined with 5 mL of ACS (Amersham) and organic samples were combined with 5 mL OCS (Amersham).

Calculations and Statistical Analysis

All values are reported as means ±1 SE mean. Each measurement for each preparation was performed in duplicate, and these values were averaged to give a single sample value. The rates of production of the various Phase I and II metabolites ($\mu g \cdot g^{-1}$ cell wet $wt \cdot h^{-1}$) were calculated using the specific activity of BaP, the wet weight of cells added, and the disintegrations per minute (dpm) (minus control dpm) in the various fractions. Total radioactivity in the eluate collected under each peak of fluorescence was used for calculations or rates of production because the various Phase I metabolites of BaP have differential fluorescence efficiencies (Schoor 1984). Significant differences (at the $p<0.05$ level) in the means of acute and acclimatory temperature treatments were tested for using a Model III, two-factor Anova and Student-Newman-Keuls test (Zar 1974). This test, which is analogous to the paired *t*-test used for comparing two treatment groups, is appropriate for the randomized block, multifactorial design of our experiments (see, for example, Walsh et al. 1988). In this test, between-preparation variation (which is significant in many cases) is assigned as the random second factor (Zar 1974).

Results

Hepatocyte Viability and Methodological Considerations

When toadfish livers are subjected to the collagenase perfusion described above, hepatocyte yield is in excess of 50%, and preparations have greater than 95% viability (typically >99%) as judged by trypan blue exclusion at the beginning and end of an experiment. Toadfish hepatocyte preparations appear to be largely biliary cells and have minimal contamination by pancreatic cells because toadfish have Brockman bodies located separately on the hepatic portal veins. In short-term incubations (i.e., ≤2 h), the toadfish hepatocyte preparation has high and stable ATP content, and exhibits rates of carbohydrate and nitrogen metabolism comparable with values obtained with hepatocytes from other fish species and to values for in vivo metabolic rates (Moon et al. 1985; Walsh 1987; Mommsen and Walsh 1989; Walsh et al. 1989). Several solvents were tested for dissolution of BaP in hepatocyte medium including acetone, methanol, ethanol, and DMSO, but only DMSO provided reliable dissolution with minimal evaporation and no apparent effects on hepatocyte viability. DMSO is routinely used to deliver fluorescent dyes to toadfish hepatocytes for intracellular pH measurement (Walsh et al. 1989) and we have measured no significant effects of DMSO at these low concentrations on lactate metabolism (K. A. Gill and P. J. Walsh, unpubl. data) and BaP metabolism (results not shown). All four control protocols tested (see Materials and Methods) gave similar results.

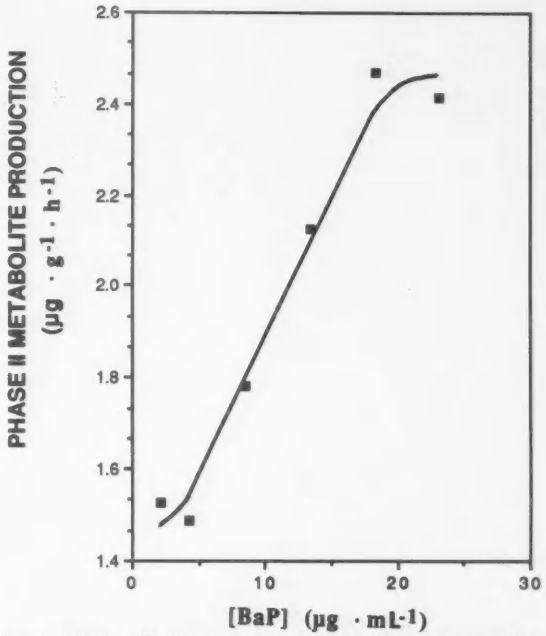

FIG. 1. Effect of [BaP] on rate of production of Phase II metabolites in toadfish hepatocytes. Values are means of duplicate experiments with male fish. Acclimation temperature = 28°C, exposure temperature = 23°C.

The metabolism of BaP to aqueous soluble metabolites was linear up to 2 h at all temperatures, so 1.5 h was routinely used in all experiments. Metabolism of BaP to aqueous soluble metabolites showed saturation at concentrations near 18 $\mu g \cdot mL^{-1}$ (Fig. 1), i.e., at a value similar to that for rainbow trout hepatocytes (Andersson and Koivusaari 1986).

Identification and Quantification of Phase I and II Metabolites

To make results of the present study more comparable with our prior in vivo results (Kennedy et al. 1989a,b) in most experiments we incubated hepatocytes at a relatively low BaP concentration (5.0 $\mu g \cdot mL^{-1}$) in comparison with saturating values (Fig. 1). Under these conditions, four major primary or Phase I metabolites of BaP were distinguished in organic extracts of toadfish hepatocytes (Fig. 2). The HPLC retention times of these four peaks coincided closely with four standards of BaP metabolites (of about 20 common BaP metabolites screened)

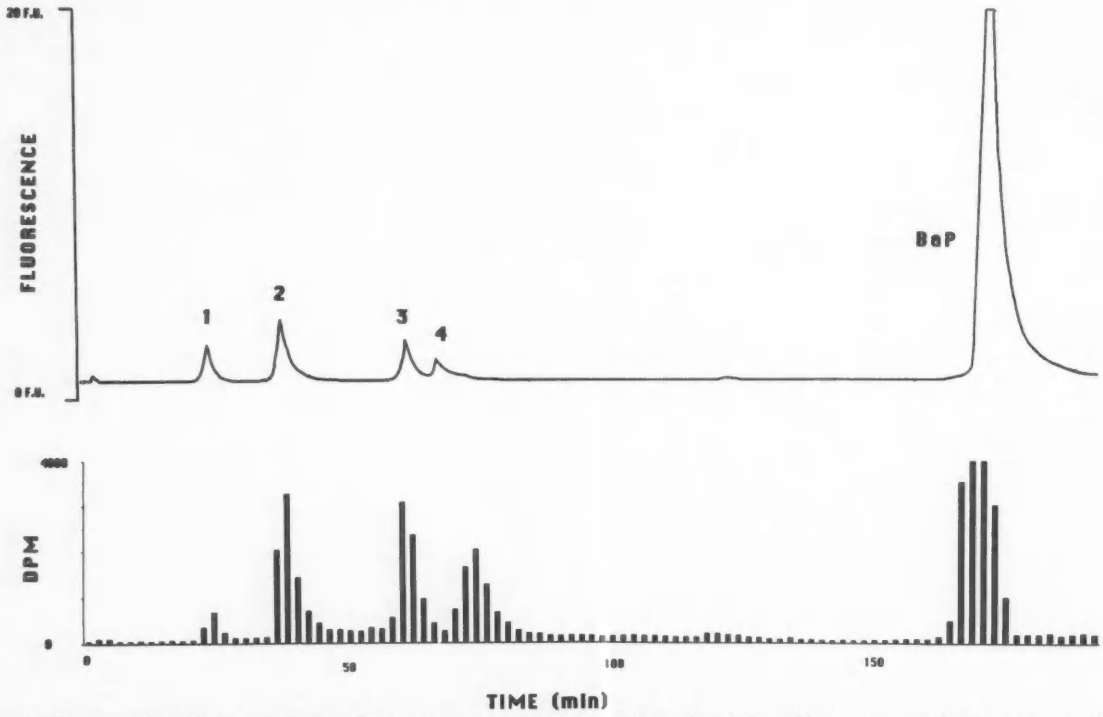

FIG. 2. Representative HPLC chromatogram (fluorescence and radioactivity profiles) of methylene chloride extract of hepatocyte suspension incubated with [^{14}C]-BaP. Peaks corresponding to numbers 1–4 are tentatively identified as (1) *r*-7, *t*-8,9, *c*-10 tetrol, (2) *t*-9,10 diol, (3) *t*-7,8 diol and (4) 4,5 dione of BaP, and peak 5 is parent BaP remaining. Note that radioactivity peak for parent BaP is truncated for diagrammatic purposes and that typically in excess of 1.5×10^6 dpm (70% of initial dose) were recovered.

TABLE 1. Retention times (min) of BaP metabolite standards and unknown metabolites from toadfish hepatocyte suspensions exposed at 5 μg(1 μCi)BaP mL^{-1}. Values are means ± SE mean. For standards N = 3 to 6; for unknowns N = 12 for each peak and incubation temperature representing pooled data from both 18 and 28°C acclimation groups.

	Exposure temperature			
Peak number	18°C	23°C	28°C	Identity
1	24.9±0.7	24.5±0.7	25.1±0.6	*r*-7, t-8,9, *c*-10 tetrol
2	39.0±0.4	37.8±0.5	38.5±0.5	*t*-9, 10 diol
3	62.4±0.5	61.6±0.8	61.7±0.8	*t*-7, 8 diol
4	70.1±0.9	70.9±1.0	70.4±0.9	4,5 dione
		Phase II Metabolite Residues		
Glucuronides	65.7±0.9	64.7±0.8	66.1±0.7	?
Sulfates	74.4±1.1	73.7±1.0	74.6±0.9	1,6 dione
		Standards		
		25.2±0.4		r-7, *t*-8,9, *c*-10 tetrol
		38.2±0.9		*t*-9,10 diol
		63.7±1.2		*t*-7,8 diol
		67.2±1.1		4,5 dione
		73.8±2.0		1,6 dione

(Table 1), and in several samples, co-injection of samples and standards produced coincident peaks. Therefore we have tentatively identified them as: *r*-7, *t*-8,9, *c*-10 benzo[*a*]pyrene tetrahydrotetrol; *t*-9,10 benzo[*a*]pyrene dihydrodiol; *t*-7,8 benzo[*a*]pyrene dihydrodiol; 4,5 benzo[*a*]pyrene dione (Table 1). However, in attempting to quantify the amounts of these compounds produced, the control values for the last peak, the 4,5 dione, were consistently as high as the values for cell sus-

TABLE 2. Effect of incubation temperature on rate of Phase I BaP metabolite production by hepatocytes isolated from toadfish acclimated to 18°C (all males) or 28°C (one female, five males) and exposed to 5 μg(1 μCi)BaP mL^{-1}. Values are means ± SE mean, $N = 6$ for each group.

Acclimation group	Rate of production (μg BaP. g^{-1} hepatocytes. h^{-1}) Exposure temperature 18°C	23°C	28°C	Metabolite
18°C	0.291 ± 0.080	0.334 ± 0.092	0.340 ± 0.114	*r*-7, t-8,9, *c*-10 tetrol
	1.840 ± 0.579	1.901 ± 0.582	2.106 ± 0.795	*t*-9,10 diol
	1.665 ± 0.458	1.469 ± 0.403	1.604 ± 0.521	t-7,8 diol
28°C	0.138 ± 0.058	0.225 ± 0.055	0.113 ± 0.042	*r*-7, t-8,9, c-10 tetrol
	1.417 ± 0.547	3.061 ± 0.548	1.838 ± 0.569	*t*-9,10 diol
	1.557 ± 0.519	2.364 ± 0.448	1.631 ± 0.548	*t*-7,8 diol

TABLE 3. Effect of incubation temperature on rate of Phase II BaP metabolite production by hepatocytes isolated from toadfish acclimated to 18°C or 28°C and exposed to 5 μg(1 μCi)BaP. mL^{-1}. Values are means ± SE mean, $N = 6$ for each group, sexes as in Table 2.

Acclimation group	Rate of production (μg BaP. g^{-1} hepatocytes. h^{-1}) Exposure temperature 18°C	23°C	28°C	Metabolite
18°C	2.04 ± 0.79	2.46 ± 0.90	2.39 ± 1.01	Glucuronides
	0.55 ± 0.24	0.42 ± 0.11	0.72 ± 0.29	Sulfates
	16.14 ± 6.17	23.18 ± 7.57	14.08 ± 4.03	Unknown
28°C	2.45 ± 0.70	3.41 ± 0.71	2.23 ± 0.72	Glucuronides
	0.48 ± 0.13	0.62 ± 0.14	0.41 ± 0.15	Sulfates
	14.23 ± 3.06	21.97 ± 4.27	15.2 ± 4.22	Unknown

pensions. This peak was not detected in HPLC analysis of the stock BaP, but it did appear in suspension medium without cells, so we concluded that the 4,5 dione was produced acellularly by uncatalyzed oxidation of BaP. Therefore, toadfish hepatocytes apparently accumulated only three Phase I metabolites in substantial quantities.

Hydrolysis of glucuronide and sulfate conjugates of BaP and subsequent HPLC analysis of the remaining organic extract each revealed only one major peak (Table 1). The retention time of the sulfate conjugate residue was close to that for the 1,6 dione and could be tentatively identified as such (Table 1). However, the retention time of the glucuronide conjugate residue was approximately halfway between the 4,5 dione and the *t*-7,8 dihydrodiol and could not be positively identified from our data (Table 1).

Phase I metabolites of BaP were accumulated at comparable rates (0.113 to 3.06 $\mu g \cdot g^{-1}$ wet wt hepatocytes$\cdot h^{-1}$, Table 2) compared with in vivo studies of toadfish (Kennedy et al. 1989a,b). Of the three major Phase I metabolites of BaP produced by toadfish hepatocytes, the one produced in greatest quantity was the *t*-9,10 diol followed by the *t*-7,8 diol and the *r*-7, *t*-8,9, *c*-10 tetrahydrotetrol (Table 2). As in prior in vivo studies (Kennedy et al. 1989a,b) the rate of accumulation of total Phase II metabolites (17.16 to 32.68 $\mu g \cdot g^{-1}$ wet wt hepatocytes$\cdot h^{-1}$, Table 3) was substantially higher than the rate of accumulation of Phase I metabolites and the largest component of the Phase II metabolites was an unknown metabolite, presumably a glutathione conjugate (Stein et al. 1987), followed by glucuronides and sulfates (Table 3).

Effect of Temperature and Metabolism of Benzo[*a*]pyrene

At the relatively low BaP concentration employed in these studies (i.e., 5 $\mu g \cdot mL^{-1}$) neither acclimation nor incubation temperature appeared to alter the types of Phase I and II metabolites produced by hepatocytes (Tables 1–3). While there was possibly a trend towards an increase in rate of production of Phase I and II metabolites between 18 and 28°C (or in some cases an optimum at 23°C), none of the effects of the temperature treatments were statistically significant (Tables 2 and 3). Since the data for the two acclimation groups were not significantly different and since substantial variation was encountered in these experiments, we also tested for an effect of exposure temperature using the pooled data for the two acclimation groups (i.e., $N = 12$); again, we found no significant effects of exposure temperature. In the limited group examined, there were no obvious effects of sex or body size on patterns of BaP metabolism.

To determine if the temperature independence noted above for metabolism of BaP at low BaP concentration extended to BaP metabolism at higher BaP concentrations we measured the effects of exposure temperature on rate of accumulation of total Phase II metabolites in hepatocytes isolated from toadfish acclimated to 18 or 28°C and exposed to higher BaP concentration. At higher BaP concentration, increasing exposure temperature had a significant effect on rates of metabolism, and rates of metabolism were significantly elevated in hepatocytes from cold-acclimated toadfish when compared at 28°C (Fig. 3).

Discussion

When hepatocytes from the gulf toadfish, were incubated with the carcinogen benzo[*a*]pyrene in short-term (i.e., ≤2 h) suspensions, BaP was rapidly metabolized to a variety of Phase I and II metabolites (Fig. 1, Tables 1–3). However, the pattern

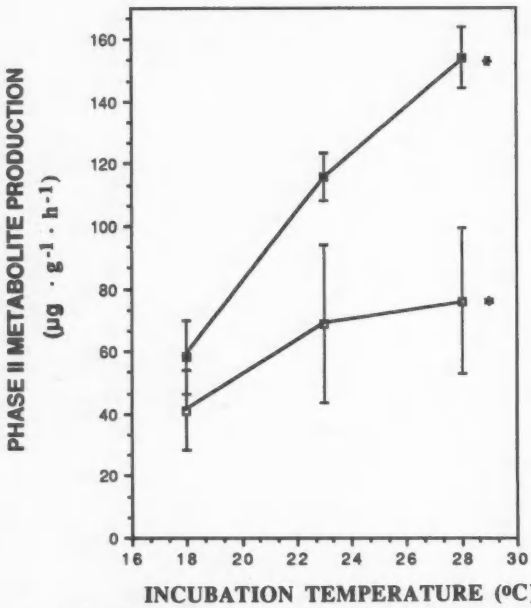

FIG. 3. Effect of incubation temperature on rate of Phase II metabolite accumulation in hepatocytes isolated from 18°C-acclimated (all males) (solid squares) or 28°C-acclimated (one female, two males) (open squares) toadfish. Initial [BaP] = 13.1 ± 0.1 μg·mL^{-1}. Values are means ± SE mean. ($N = 3$). There is a significant effect of incubation temperature in both groups, and the rates of metabolism at 28°C are significantly different between acclimation groups (asterisks) ($p < 0.05$). Q_{10} values are 1.85 (18°C-acclimated) and 2.65 (28°C-acclimated).

of metabolites produced was slightly different from the metabolites produced in vivo in prior studies by others (Varanasi 1989) and in our laboratory (Kennedy et al. 1989b). We could detect only three major Phase I metabolites (tentatively identified as *r*-7, *t*-8,9, *c*-10 tetrahydrotetrol; *t*-7,8 diol; *t*-9,10 diol) that accumulated in the hepatocyte suspensions (Fig. 1; Table 1), whereas after a 24-h exposure in vivo, toadfish accumulated at least 10 other identifiable metabolites of BaP including tetrols, triols, diols, diones, and phenols (Kennedy et al. 1989b). However, the three Phase I metabolites identified in hepatocyte suspensions were also the three most abundant Phase I metabolites produced in vivo (Kennedy et al. 1989b). The difference between the two studies may simply be due to the shorter exposure time in vitro, and our inability to detect presumably lower concentrations of some Phase I metabolites. However, an alternative possibility is that other tissues are producing Phase I metabolites of BaP in vivo. Studies of BaP metabolism with hepatocytes in longer-term culture, and of BaP metabolism by other cell types in vitro would help to distinguish between these possibilities.

Despite these in vivo and in vitro differences in BaP Phase I metabolite accumulation, Phase II metabolites were the major BaP metabolites produced in vitro (Table 2 and 3), as has been shown in fish in vivo (Varanasi 1989; Kennedy et al. 1989b). Phase II metabolites of BaP accumulated in toadfish hepatocyte suspensions in approximately the same relative proportions as in the bile in vivo, that is, mostly as an unidentified aqueous compound, with a smaller percentage as glucuronide and sulfate conjugates (Table 3; Kennedy et al. 1989b).

At concentrations of BaP approaching saturation of detoxification pathways, effects of temperature on rates of Phase II metabolite accumulation were substantial (Fig. 3). Increasing exposure temperature significantly increased rates of BaP metabolism, although there was some independence from temperature at higher temperatures in hepatocytes from the warm acclimated fish. There was a significant effect of acclimation temperature, and these results fit a pattern of "rotation" (Prosser 1973), that is, the warm acclimated curve is rotated downward such that, following acclimation, the rate at 28°C is nearly identical to the rate at 18°C. The effects of acute and acclimatory temperature changes on BaP metabolism in toadfish hepatocytes at high BaP concentration were similar to those observed for BaP metabolism by rainbow trout hepatocytes by Andersson and Koivusaari (1986). The adaptive significance of such acclimatory responses has been interpreted as follows: when temperature increases are long-term (i.e., seasonal) the organism takes advantage of the increase in rates of metabolism due to the direct effects of temperature on enzyme reaction rates, and thus it does not need to maintain a higher (and energetically speaking presumably more costly) titre of detoxification enzymes (Prosser 1973; Hochachka and Somero 1984). However, in this regard, studies of the acclimation effects on activities of detoxification enzymes have produced mixed results; acclimation temperature increases have produced no change, increases, or decreases in enzyme activities depending upon the enzyme or species studied (e.g., Stegeman 1979; Koivusaari 1983; Karr et al. 1985; Lindstrom-Seppa 1985; Andersson and Koivusaari 1985). Clearly, further studies of the enzymic mechanisms of the acclimation response are necessary in systems in which effects of temperature on metabolism have been characterized.

Our prior in vivo studies of the effects of temperature on uptake and metabolism in toadfish at low BaP concentrations suggested that high temperatures led to the accumulation of higher amounts of the most carcinogenic BaP metabolites, the BaP diol epoxides, as judged by the relative proportions of diol epoxide breakdown products, the BaP tetrols and triols (Kennedy et al. 1989b). Unfortunately, since far fewer Phase I metabolites accumulated in our short-term in vitro incubations (Table 1), our in vitro results do not allow us to further examine the mechanisms of these effects in vivo. However, our in vitro results are important to a second tentative conclusion reached in our in vivo studies, that the overall rate of metabolism of BaP to Phase II metabolites may be relatively temperature insensitive under some conditions. In vivo, most of the effect of temperature on the rate of accumulation of BaP metabolites in the bile could be accounted for by the effect of temperature on rates of uptake alone (Kennedy et al. 1989a,b). Although it is difficult to separate uptake and metabolism in vivo, our in vitro results appear to confirm our prior conclusion. At low BaP concentrations relative to saturation values (Fig. 1), but higher than those employed in vivo, variation of acclimation and exposure temperature had no statistically significant effect on types of Phase I and II metabolites produced (Table 1) nor on rates of accumulation of these metabolites (Table 2 and 3) in toadfish hepatocytes. Considerable variation between individuals was encountered in these experiments, but fish hepatocyte preparations typically have high individual variation (Moon et al. 1985). In the case of carbohydrate metabolism, for example, this variation is reflective of highly variable endogenous gly-

cogen stores. This type of variation can normally be factored out by the paired experimental design and Model III, two-way Anova described in the Materials and Methods. Typically, sample sizes of six to seven fish per group are adequate to detect significant differences of 25% between means (see Walsh et al. 1988). No gross patterns of differences in BaP metabolism with sex or size of fish were evident. Although the high variability of our results could reflect differences in prior exposure to xenobiotics in the environment, this is unlikely since the toadfish all came from the same area of south Biscayne Bay which borders on a marine sanctuary, and which is noted for relatively low sediment PAH content (less than 0.7 μg/g sediment, total PAH) (Corcoran et al. 1983).

What are the possible mechanisms of the observed temperature independence of BaP metabolism at low substrate concentration? For virtually all enzymes of intermediary metabolism studied, the K_m for substrate either increases or remains constant with temperature (Hochachka and Somero 1984). Thus, as reaction rates increase in response to acute temperature increases, given a constant subsaturating substrate concentration, an increase in K_m or decrease in enzyme affinity has the potential to offset this increase in rate; this phenomenon has been termed "positive thermal modulation" (Hochachka and Somero 1973). To our knowledge, the effects of acute temperature changes on the kinetic characteristics of the enzymes of xenobiotic detoxification have not been studied in any great detail. These types of studies may provide a mechanistic explanation for our in vitro and in vivo observations of low temperature sensitivity of BaP metabolism at low BaP concentrations. Finally, in view of this low temperature sensitivity of BaP metabolism, under some circumstances (e.g., low carcinogen concentration) the observed effects of temperature on carcinogenicity may be more related to effects of temperature on other steps (e.g., uptake) in the processing of carcinogens.

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Cyclic Dominance and Optimal Escapement of Adams River Sockeye Salmon (*Oncorhynchus nerka*)

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Welch, D. W., and D. J. Noakes. 1990. Cyclic dominance and optimal escapement of Adams River sockeye salmon (*Oncorhynchus nerka*). Can. J. Fish. Aquat. Sci. 47: 838–849.

We examined escapement policies for a stock-recruitment model with negative between-year interactions. Regardless of the degree of interaction present, the optimal policy is to always equalize escapement. Parameter estimates obtained for the Adams River sockeye salmon (*Oncorhynchus nerka*) indicate that between-year interactions may occur, but confidence regions include the null hypothesis of no interaction at all (the Ricker model). We conclude that the extreme amplitude of the current recruitment cycle in this stock frustrates statistical identification of interaction. It seems unlikely that between-year interactions will be measurable until the off year runs increase by at least two to three orders of magnitude. Comparison of total yields for the Adams River sockeye shows that an equal escapement policy could increase yields by at least 35% over that obtained by the current cyclic escapement pattern. This is equivalent to obtaining an additional $27 million in total yield per annum from the Adams River stock alone and, assuming a discount rate of 4%, translates into an increase in net present value of $675 million. If between-year interactions do not exist, the potential benefits of moving to an equal escapement policy are even larger, on the order of $3 to $4 billion.

Notre étude examine les politiques de gestion relatives à l'échappée au moyen d'un modèle de recrutement des stocks prenant en considération les interactions négatives pouvant exister au sein des stocks d'année en année. Quel que soit l'importance de ces interactions, la meilleure politique consiste toujours à faire en sorte que l'échappée soit la même à chaque année. Selon nos estimations portant sur le saumon nerka (*Oncorhynchus nerka*) de la rivière Adams, il pourrait y avoir interaction interannuelle dans ce stock, mais les régions de confiance montrent qu'il est possible qu'il n'y en ait aucune (modèle de Ricker). Nous en concluons qu'il est impossible d'établir au moyen de méthodes statistiques l'existence d'interactions interannuelles en raison de la trop grande amplitude du cycle de recrutement actuel de ce stock. Il semble improbable qu'il soit possible de mesurer ces interactions tant que le nombre de saumons de remonte dans les années de faible montaison n'aura pas augmenté d'au moins deux ou trois ordres de grandeur. Selon notre analyse des rendements totaux du stock de saumon nerka de la rivière Adams, l'adoption d'une politique visant à rendre l'échappée constante d'année en année pourrait augmenter les rendements d'au moins 35 % par rapport à ceux obtenus sous le régime actuel où les échappées varient de façon cyclique. La production totale du stock de la rivière Adams pourrait ainsi être augmentée de 27 millions de dollars par année, ce qui représente une augmentation de la valeur actualisée nette de 675 millions de dollars et ce, si l'on suppose un taux d'actualisation de 4 %. S'il n'y a pas d'interaction interannuelle, l'adoption d'une politique visant à maintenir l'échappée constante d'année en année pourrait augmenter la valeur de cette pêche de 3 à 4 milliards de dollars environ.

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The numbers of sockeye salmon (*Oncorhynchus nerka*) returning to many tributaries of the Fraser River exhibit strong cyclical fluctuations, with the pattern of returns repeating every 4 yr (Ricker 1950). Although the year of peak returns to many stocks shifted following the Hell's Gate rock slide of 1913 that decimated many upper Fraser runs, the stability of this cycle is nevertheless remarkable. Historical records suggest that a 4-yr-cycle in the Fraser was present at least as far back as 1793 (Ward and Larkin 1964), and returns of the Adams River stock in this century provide a particularly dramatic example of cyclic behaviour. Since 1948, when quantitative data first became available, recruitment has varied regularly in a cycle with mean ratio of 474:69:1.5:1, or very roughly an order of magnitude decline between successive years within the cycle.

Despite considerable interest, the causes of this phenomenon remain uncertain. Biologically based hypotheses suggest that negative interactions between different runs to the system perpetuate it (Ricker 1950; Johnson 1965; Ward and Larkin 1964; Larkin 1971). A more recent suggestion is that higher harvest rates in years of weak returns helped create and maintain the observed cycle (Walters and Staley 1987). Whatever the cause, the cyclic behaviour of sockeye returns to the Fraser River system is currently of intense interest because of plans to increase the total yield of Fraser sockeye by increasing escapement levels (Collie and Walters 1987; Walters and Staley 1987; unpublished background papers of the Fraser Task Force Cyclic Dominance Working Group). However, if the cycle in Adams sockeye returns is maintained by negative interaction between different runs to the system, the possibility exists that increasing escapement to the weak runs might reduce the overall productivity of the system.

These concerns are hardly novel. Ricker (1950), although clearly believing in the existence of biological interactions, noted that "It is not clear why there should be [just] one big cycle... two would seem quite feasible, occurring in alternate years" (p. 16). He subsequently noted that "... there is certainly a possibility that a greater sockeye yield could be obtained if

each lake had four more or less equal cycles" (p.22), although he felt that it was more feasible to make the recruitment cycles for different populations peak in different years of the cycle rather than to move to an equal escapement policy for each population.

Uncertainty about the effect different management policies might have on the overall productivity of the system continues to haunt management of the Adams River stock. As Ward and Larkin (1964, p.3) stated, "Without knowledge concerning the causes of... cyclic dominance, management procedures actually designed to increase average quadrennial catches might result in a reduction rather than an increase." Given the value of the Adams River stock, and the continuing failure to pin down the mechanisms causing cyclic dominance, it is hardly surprising that managers have been wary of changing the historical pattern of returns, and have been content to manage the stock within this cyclical framework.

Indeed, when the return of the subdominant sockeye run to the Adams River in 1971 proved to be larger than that of the preceding dominant year, it was deliberately fished down to preserve the historical ratio of escapements, precisely because of concerns about the biological nature of cyclic dominance (IPSFC 1972, p.25). The risk of destroying the dominant run clearly outweighed the possible benefits from having two large runs, even though computer simulations of the day suggested that the latter situation seemed to be stable, and unlikely to seriously affect the productivity of the dominant run (Larkin 1971).

Determining the nature of this cyclic behaviour is therefore far from strictly academic interest. The high value and large numbers of sockeye returning to the Fraser system mean that different management schemes have radically different economic implications, and that the best management strategy may depend on identifying the underlying mechanisms. If cyclic behaviour is due entirely to differential between-year harvest rates rather than biological interactions, each run within the 4-yr-cycle is independent (Walters and Staley 1987). As the dominant run is nearly 500 times larger than the off-year runs, the absence of interactions means that escapement to the off-year runs is 500 times too small.

On the other hand, increasing the size of smaller runs will decrease the productivity of the dominant run if negative interaction does occur. Whether the economic benefits achieved by increasing the size of the small runs can compensate for the decreased productivity of the dominant run is unclear in this case. These concerns clearly underlie some of the management actions taken in the past. However, an implicit assumption made here is that the observed cycle provides near-optimal production when negative interactions do exist.

Resolution of whether some form of cyclical pattern of returns gives the best yield also has implications beyond the Adams sockeye. "Cyclical" returns to other sockeye populations, both within and without the Fraser, also occur. However, the shape of these patterns has little in common with that of the Adams River cycle, apart from the 4-yr periodicity. Sequential returns to some populations within the Fraser build to a peak before sharply falling, (the opposite of the pattern for the Adams stock), while in other populations the patterns are erratic, but exhibit persistence with time. Returns of pink salmon (*Oncorhyncus gorbuscha*) are even more striking, with returns in southern British Columbia occurring only in odd calendar years — a 2-yr-cycle. The question of what role density-dependent interactions play in determining the best management strategy is equally germane for these populations.

A satisfactory biological explanation of cyclic dominance seems likely to emerge only when these alternative patterns are accounted for as well. Given the difficulty involved in disproving the existence of some postulated form of biological interaction in the field, it seems fruitful to attack the problem from the opposite viewpoint: whether between-year interactions can be detected statistically, and what management strategy leads to the optimal yield in the presence of interactions. These are the points we address below.

Cyclic Dominance Models

It is far from clear what policy should be best if interactions do occur, or indeed whether there could be several different policies, each optimal for some level of interactions. Collie and Walters (1987) employed stock and recruitment data for the Adams River population to test the adequacy of two stock–recruitment models, and to examine the predicted productivity of the models. These authors estimated the stock–recruitment parameters for the Ricker model (Ricker 1954) and a model proposed by Larkin (1971) that incorporates interaction terms to account for the effect of predators or competitors. Larkin's model has the form

$$(1)\quad R_t = S_t \exp(a - b_0 S_t - b_1 S_{t-1} - b_2 S_{t-2} - b_3 S_{t-3} + \sigma\epsilon_t),$$

where R_t is recruitment at time t, S_t is the stock size causing recruitment at time t, a, b_0, b_1, b_2, b_3, and σ are estimated parameters, and ϵ_t is an error term assumed to be normally, identically, and independently (NIID) distributed with mean zero and variance one. The b_i parameters represent first order dependencies in the system that negatively affect recruitment in year t. Larkin considered the use of stock size in previous years, S_{t-i}, as surrogates to account for the effects of predators or competitors in the system whose abundance rises in response to increases in sockeye recruitment. When only a and b_0 are nonzero, equation (1) reduces to the Ricker model.

Intuitively, one would expect the influence of previous stocks to attenuate with time. That is, S_t should presumably have a greater influence on R_t than S_{t-i}, $i=1,2,3$, and should be reflected in smaller values of b_i with increasing i. Collie and Walters (1987) reported this general trend with one notable exception. They found b_2 was larger than b_1 and nearly as large as b_0, indicating that stock size in year $t-2$ was almost as important as stock size in year t. As no confidence intervals were reported, it is uncertain whether the variation in the parameter estimates is statistically significant. In any case, it is difficult to clearly establish the effect of lagged density-dependence on productivity with four different terms present. Collie and Walters found numerically that between-year density-dependence could significantly reduce the overall productivity, but exactly how productivity depends on density-dependence is unclear in the Larkin model.

We propose a model similar to Larkin's in this paper, which incorporates inter-year dependencies but involves fewer parameters. Our proposed stock–recruitment model has the form

$$(2)\quad R_t = \alpha S_t \exp\left\{-\beta \sum_{i=0}^{\infty} \rho^i S_{t-i}\right\} = \alpha S_t \exp\{-\beta(S_t + \rho S_{t-1} + \rho^2 S_{t-2} + \ldots)\},$$

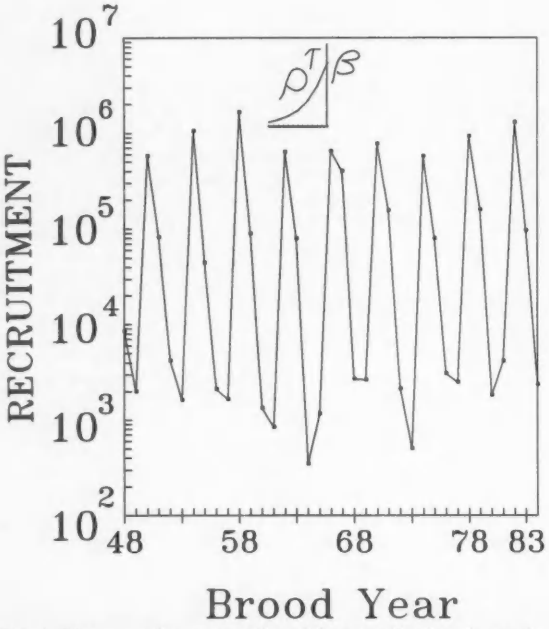

FIG. 1. Diagrammatic representation of the generalized stock–recruitment model described in the text. The measure of stock used to estimate interaction is defined as the sum of past escapements, but with each unit of stock given weight β, and spawners τ-yr prior discounted at the rate ρ^τ (see inset). Note that stock size is defined in numbers, but the logarithm of the number of recruits has been plotted to clearly show the recruitment cycle.

where $0 \leq \rho \leq 1$ is a parameter used to model the inter-year dependence. By varying the magnitude of ρ, we can change the window defining how previous spawning stocks contribute to the measure of lagged stock (Fig. 1). This stock–recruitment model, generalized to include inter-year density-dependent interactions, is an example of a transfer function time series model (Box and Jenkins 1976; Noakes et al. 1987).

Two major biological assumptions underlying the use of this model are that density-dependence is related to current and past spawning stock sizes, and that the per capita influence on survival of each additional spawner is equal and independent of cycle year. We also implicitly assume that those aspects of the system influencing sockeye survival recuperate exponentially after perturbation (e.g. following grazing of the lake's zooplankton population) so that the per capita influence of stock in year $t-1$ is some fraction ρ of the influence in year t.

To be more specific, we assume a constant per capita density-dependent mortality coefficient β ($0 \leq \beta < \infty$), but one where the influence of spawners τ years prior is discounted at the rate ρ^τ. Note that when $\rho = 0$ (no between-year density-dependent interactions) the model reduces to the standard Ricker curve, since we define $\rho^0 = 1$ when $\rho = 0$. When $\beta = 0$ the model collapses to the simplest possible model of no density-dependence at all. Biologically plausible parameter values therefore lie in the region $\beta \geq 0$, $0 \leq \rho < 1$.

Our generalized model differs from the Larkin model used by Collie and Walters (1987) in several respects. First, the model has an infinite memory, but one which involves the estimation of only four parameters (α, β, ρ, and the residual variance σ^2) as opposed to six for the Larkin model (a, b_0, b_1, b_2, b_3, and σ^2). This sidesteps the need to specify a priori an arbitrary time limit on the persistence of past effects, and allows the data to determine the number of years of past spawning stock sizes effectively included in the model. As the available data are limited (we used data from brood years 1946–84 ($N = 37$)) this flexibility is an important consideration. Second, the imposition of some order on the density-dependent terms by requiring a regular decline with time is intuitively reasonable and statistically testable. And third, the relatively simple form of our generalized model (2) allows derivation of the optimal stock sizes and ensuing yields as a function of only two parameters, each with clearly distinct biological meaning. This leads to considerable insight about the production dynamics of the Adams River stock.

Before turning to the problem of estimating the parameters in equation (2), we first consider the general problem of determining which recruitment pattern(s) give the optimum yield, and whether the optimal management policy changes depending on the magnitude of the density-dependent interaction terms.

Optimal Recruitment Patterns

Analytic Results

Achieving optimal economic yield, OEY, requires maximizing the difference between biological production and the amount of stock needed to just maintain the population. This point always occurs at a smaller stock size than that producing maximum biological production, MBP (Ricker 1975).

We have derived the expression defining the stock size providing optimal economic yield for our generalized recruitment model in Appendix B, equation (B6). However, in common with the Ricker equation this expression is nonlinear and does not give an explicit analytic form for the optimal stock size in terms of its parameters, although in practice it is simple to solve for these stock sizes numerically. In this section we have therefore chosen to report the stock sizes that achieve maximum biological production under various escapement strategies, because the analytic expressions make the effect of between-year interaction on suppressing total yields and stock sizes clear. We report the stock sizes yielding the maximum economic return, and the level of returns achieved, in a later section. It turns out that both the stock sizes giving optimal yield, and the level of yield achieved, are very similar to the levels giving maximum biological production when ρ is large.

The cumulative 4-yr production of sockeye salmon returning to the Adams river, $\psi(S)$, is simply the sum of recruitment, over the 4-yr of the cycle:

$$(3) \quad \psi(S) = \alpha \sum_{i=1}^{4} S_i \exp\left\{-\beta \sum_{t=0}^{\infty} \rho^t S_{i-t}\right\} .$$

At maximum biological production, stock size in each of the 4-yr of the cycle is constant (although not necessarily equal), such that $S_i = S_{i+4n}$, where n is an integer. This observation, plus the fact that $\sum_{t=0}^{\infty} \rho^t = (1-\rho)^{-1}$ for $|\rho| < 1$, allows us to simplify equation (3) to the more compact form:

$$(4) \quad \psi(S) = \alpha \sum_{i=1}^{4} S_i \exp\left\{\frac{-\beta}{1-\rho^4} \sum_{t=0}^{3} \rho^t S_{[i-t]}\right\}$$

where $\psi(S)$ is the cumulative production, and S_i the spawning

stock size in year i. Expressions in square brackets, such as the subscript $[i-t]$, obey modula$_4$ arithmetic, such that

$$(5)\quad [i-t] = \begin{cases} i-t, & i > t \\ i-t+4n, & i \leq t, \end{cases}$$

where n is chosen as the smallest positive integer sufficient to make $[i-t]$ positive.

Given α, β, and ρ, maximum biological production (i.e. the peak of the stock–recruitment curve) is calculated by finding the set of spawning stock sizes, $S_{i,MBP}$, $i=1,\ldots4$, such that the first partial derivatives with respect to stock size equals zero for all S_i. Taking the first partial derivative of total yield over the 4-yr-cycle with respect to S_j, yields

$$(6)\quad \frac{\partial\psi(S)}{\partial S_j} = \alpha\left(\exp\left\{\frac{-\beta}{1-\rho^4}\sum_{t=0}^{3}\rho^t S_{[j-t]}\right\} - \frac{\beta}{1-\rho^4}\sum_{i=1}^{4} S_i\rho^{[i-j]}\exp\left\{\frac{-\beta}{1-\rho^4}\sum_{t=0}^{3}\rho^t S_{[i-t]}\right\}\right)$$

A maximum (or minimum) exists when all four derivatives $\partial\psi(S)/\partial S_j$, $j=1,..,4$, are equal to zero.

Because of the complicated nonlinear nature of $\partial\psi(S)/\partial S_j$, it is not immediately clear how many combinations of stock sizes S_j, $j=1,..,4$ make all the first partial derivatives (equation (6)) simultaneously zero. The absolute minimum occurs when all stocks are equal to zero, but other extreme points (maximum or minimum) may also exist. In fact, a general truism about nonlinear equations is that they may have more than one solution (i.e. set of stock sizes) that satisfy the optimality condition.

Simply finding one solution is therefore no assurance that some other combination(s) of stock sizes does not exist that also leads to a local maximum. Complicating matters further, there is no guarantee that a particular solution is the global maximum or minimum. To determine whether an extreme point is locally or globally maximal requires evaluating each solution individually.

It is easy to show for the Ricker equation that the only solution leading to a production maximum occurs at $S_{MBP} = 1/\beta$. We cannot prove analytically that there is only one combination of stock sizes that yields the maximum productivity to our generalized model. However, we found that numerical minimization of equation (6) always led to the same solution; namely, that escapement to each of the four cycles should be equal, irrespective of the combination of stock sizes used to start the search. This is the same empirical finding reported by Collie and Walters (1987) for the Larkin model, using the coefficients they estimated from the Adams River data. We now analytically derive the stock sizes giving maximal production for several other biologically interesting recruitment patterns. After doing this, we can compare the relative stock sizes and productions predicted by these models for the Adams River stock, and relate their dependence to the model parameters.

Case I: equal stock sizes

If we make the leap of faith that one solution is $S_i = S_{MBP}$, $i=1,..4$, then it is possible to show (Appendix A) that the solution is

$$(7)\quad S_{MBP} = \frac{(1-\rho)}{\beta},$$

for all years of the cycle. Substitution of this value back into equation (6) shows that it does indeed satisfy the equations, and calculation of the second derivative shows that this solution is a maximum.

The major conclusions from this result are that (1) the solution to the maximal production problem reduces to the Ricker stock size solution, $S_{MPB} = 1/\beta$, in the absence of between-year density-dependence ($\rho=0$), (2) between-year density-dependence has a direct linear influence on reducing the stock size providing maximum biological production, and (3) the ratio of the two density-dependent parameters β and ρ determines the stock size giving maximum biological production, independent of α.

Maximum production for the equal stock size solution is obtained by substituting equation (7) into our generalized model's production equation (4). This yields the estimated production summed over the 4 yr of the cycle:

$$(8)\quad \psi(S)\Big|_{S_i=S_{MBP}} = \frac{4\alpha(1-\rho)}{\beta e} = \frac{1.47\alpha(1-\rho)}{\beta},$$

where $e=2.718\ldots$, the base of the natural logarithms.

By invoking certain constraints, it is possible to determine the productivity of several other life history patterns of particular interest. In particular, we consider the effect of imposing the following escapement patterns (illustrated in Fig. 2) on the Adams sockeye run.

Case II: a pink salmon (*Oncorhynchus gorbuscha*) recruitment pattern

Pink salmon returning to the Fraser River exhibit an even more striking cyclic pattern than the Adams sockeye, with essentially zero recruitment in even calendar years, and returns of many millions in the odd calendar years (e.g. 1989). If we (mathematically!) wipe out alternate sockeye runs, and determine the optimal stock size and productivity for the remaining runs, we have the sockeye equivalent of a pink salmon life history. To use an analogy from agriculture, we wish to allow the Adams River to life fallow in alternate years because of the damaging effects of constant planting when between-year density dependence occurs. In agriculture, this might be caused by a depletion of essential nutrients, or a build-up of parasites or pathogens.

Specifically, we constrain $S_2 = S_4 = 0$, and solve for the optimal stock sizes for the remaining runs, S_1 and S_3, that maximize recruitment yield over the 4-yr-cycle. The solution (Appendix A) shows that maximum biological production is obtained by setting the odd year runs to the values:

$$(9)\quad S_1 = S_3 = \frac{(1-\rho^2)}{\beta}.$$

(The same solution holds because of symmetry if the odd year returns were wiped out instead of the even year runs).

Maximum production is obtained as for Case I, but remembering to set $S_2 = S_4 = 0$:

$$(10)\quad \psi(S)\Big|_{S_1=S_3=S_{MBP}} = \frac{2\alpha(1-\rho^2)}{\beta e} = \frac{0.74\alpha(1-\rho^2)}{\beta}$$

Case III: "extreme" cyclic dominance

An "extreme" caricature of the Adams River cycle dominance pattern is to allow two consecutive years of significant returns followed by 2 yr of zero recruitment (cf. Ricker 1950). Referring to our agricultural analogy again, the strategy is to

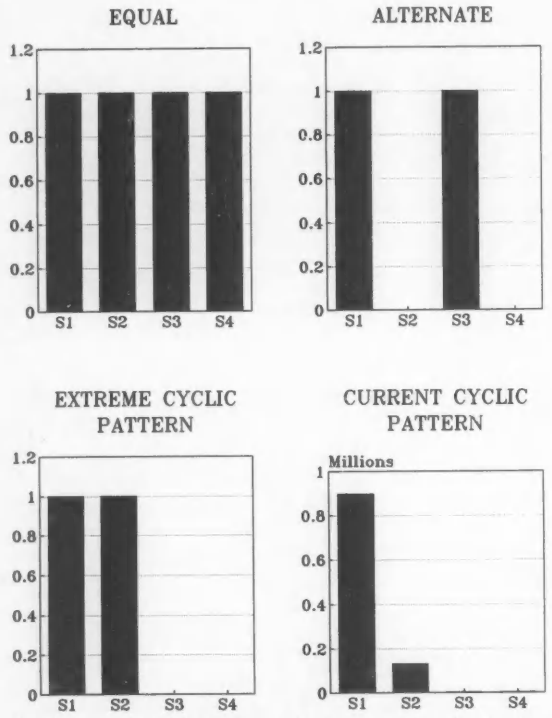

FIG. 2. Examples of the escapement patterns considered in the text. With the exception of the "Current" pattern, which shows historic mean returns, the scale of the figures is arbitrary. S1 – S4 = stock sizes in years 1 to 4 of the cycle.

calculate the stocking density leading to maximum production for the two large runs currently present within the Adams system, and to allow the system to recover by requiring it to then lie fallow for two consecutive years. This scenario is similar to the current Adams River recruitment pattern, in that 2 yr of very low returns follow the dominant and subdominant return years.

To examine this model, we again wipe out the last 2yr of the cycle, and solve for the optimal stock sizes for the first 2 yr of the cycle (i.e. we set S_1, $S_2 \neq 0$, $S_3 = S_4 = 0$).

The result (Appendix A) shows that stock sizes in the two nonzero return years will be unequal and must satisfy the following ratio:

$$(11)\quad \frac{S_1}{S_2} = \frac{1-\rho \exp\left\{\frac{\beta}{1-\rho^4}\left[(1-\rho)S_1 + (\rho^3-1)S_2\right]\right\}}{1-\rho^3 \exp\left\{\frac{-\beta}{1-\rho^4}\left[(1-\rho)S_1 + (\rho^3-1)S_2\right]\right\}}$$

This implies $S_2 > S_1$, the opposite of the dominant:subdominant pattern historically seen in the Adams River sockeye returns. Simulation studies by Larkin (1971) predicted a similar pattern of returns, with the subdominant run becoming dominant. Production is calculated by substituting the optimal stock sizes back into the production equation (4).

Case IV: "current" cyclic dominance

The historical pattern of recruitment to the Adams stock in the past half century is that the relative ratio of returning adults in successive years is roughly 474:69:1.5:1. (The size of the two smallest runs is uncertain because of the difficulty in discriminating small numbers of Adams River fish from larger comigrating stocks). If we assign relative abundances γ_i = 474:69:1.5:1 to the 4 yr of the cycle in order to control the shape of the cycle (but not its magnitude), it is possible to calculate the stocking density, S_{MBP}, needed to optimize the yield while maintaining the historic cycle. In this case, our generalized recruitment model (4) becomes:

$$(12)\quad \psi(S) = \alpha \sum_{i=1}^{4} S_{MBP}\gamma_i \exp\left\{-\beta \sum_{t=0}^{\infty} \rho^t S_{MBP}\, \gamma_{[i-t]}\right\} .$$

Taking the derivative with respect to S_{MBP}, we find that the stock size for the i^{th} year of the cycle becomes:

$$(13)\quad \gamma_i S_{MBP} = \frac{1}{\beta \sum_{t=0}^{\infty} \rho^t \gamma_{[i-t]}} .$$

Maximum biological production over the 4-yr-cycle then becomes:

$$(14)\quad \psi(S)\Big|_{S_i=\gamma_i S_{MBP}} = \alpha \sum_{i=1}^{4} \frac{\gamma_i}{e\,\beta \sum_{t=0}^{\infty} \rho^t \gamma_{[i-t]}} .$$

In practice (Appendix A), we can specify any arbitrary set of values for the γ_i, and then numerically solve for the value of S_{MBP} that leads to a production maximum in equation (14).

Estimation

So far we have only proposed a simple model of the Adams River sockeye dynamics, and posed some biologically interesting questions about the properties of the model. We now estimate the model parameters, and examine their relevance to proposals to rebuild the Adams River sockeye population. We have used stock and recruitment data for the brood years 1948–84 supplied by W. Saito (Department of Fisheries and Oceans, 503 Mentmore St., Coquitlam B.C. V3J 4P5, pers. comm.). Recruitment is in numbers (catch plus escapement), and stock is the female escapement corrected for between-year changes in fecundity and partial spawning success.

Residual variation in Pacific salmon returns, ϵ_i, is generally lognormally distributed (Peterman 1981), so our model of sockeye recruitment becomes

$$(15)\quad R_i = \alpha S_i \exp\left\{-\beta \sum_{t=0}^{\infty} \rho^t S_{i-t} + \sigma\epsilon_i\right\} ,$$

where α, β, ρ, and σ are estimated parameters, and ϵ_i is an error term assumed to be normally, identically, and independently (NIID) distributed with mean zero and variance one. Under the NIID assumption the sum of squared errors is proportional to the log-likelihood. Maximum likelihood estimates can therefore be obtained by minimizing the sum of squared errors:

$$(16)\quad \text{Min} \sum_{i=1}^{N} \epsilon_i^2 = \text{Min} \sum_{i=1}^{N} \left\{\text{Ln}(R_i/\alpha S_i) + \beta \sum_{t=0}^{\infty} \rho^t S_{i-t}\right\}^2$$

The infinite sum $\Sigma_{t=0}^{\infty} \rho^t S_{i-t}$ in expression (16) complicates

TABLE 1. Comparison of parameter estimates for our generalized recruitment model equation (2) with the Larkin and Ricker models. The final parameters reported here were estimated using a $\tau = 4$ year window for calculation of lagged stock effects (see text). Parameter estimates for the Larkin model differ slightly from those reported in Collie and Walters (1987); we used more recent data not available to those authors.

A. Initial parameter estimates[a]

Parameter	Ricker	Generalized	Larkin
α	11.82	19.17	33.4
β	7.74×10^{-7}	1.07×10^{-6}	1.65×10^{-6}
ρ	zero, by definition	0.63	see below
σ_ϵ^2	1.12	1.09	1.08

B. Lag coefficients

i	$\rho^i\beta$ ($\times 10^{-6}$)	b_i ($\times 10^{-6}$)
0	1.07	1.65
1	0.68	0.85
2	0.43	1.33
3	0.27	0.87
4	0.17	(not estimated)
5	0.11	"
6	etc...	"

[a] $\sigma_\epsilon^2 = \Sigma_i \epsilon_i^2/(N-p-1)$, where N is the number of observations, and p the number of fitted parameters.

parameter estimation since it involves estimates of stock size prior to the collection of data (i.e. $i-t \leq 0$). To rectify this situation, the stock size series was extended backwards in time using the means of each of the four cycle lines in place of the unobserved stock sizes. A cutoff value, τ, was also established to provide a reasonable approximation to the infinite lagged stock summation (review by Klipstein, 1967). The cutoff was arbitrarily set to include 90% of the total area under the curve $\Sigma_{t=0}^{\infty} \rho^t$. The infinite sum in equation (16) was therefore replaced by $\Sigma_{t=0}^{\tau} \rho^t S_{i-t}$. To minimize the influence of using the cycle means as start-up values, the first four residuals were not employed in the sum of squares calculation (16). To be explicit, we chose to minimize the expression

$$(17) \quad \sum_{i=5}^{N} \left\{ \mathrm{Ln}(R_i/\alpha S_i) + \beta \sum_{t=0}^{\tau} \rho^t S_{i-t} \right\}^2$$

with respect to the parameters (α, β, ρ) using the simplex algorithm (cf. Mittertreiner and Schnute, 1985). The maximum likelihood estimates of ρ we report below are therefore based on the sum of squares calculations starting at $i = 5$. To facilitate comparison, we set the summations to start at $i = 5$ for the Larkin and Ricker models as well, in order to match the observations available for the generalized model.

Numerical Results

Parameter estimates for the Ricker, Larkin, and generalized recruitment models (Table 1) show that the generalized model estimates an intermediate density-dependent response, with values lower than estimated for the Larkin model but with a longer tail (Fig. 3). Because we used an updated data set, our estimates of the Larkin model parameters differ slightly from those previously reported (Collie and Walters 1987), but are comparable.

The residual variance for our model is about equal to that of the Larkin model and only very slightly smaller than that of the

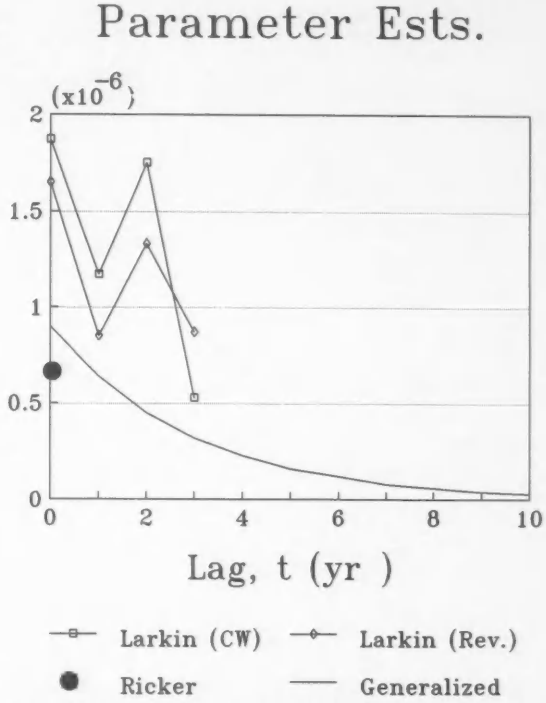

FIG. 3. Plot of the weighting function $\beta\rho^t$ defining how past stock sizes contribute to the measure of lagged stock. The figure also shows parameter estimates for the Ricker model and both Collie and Walters' (1987) and our own revised estimates for the Larkin model.

Ricker model. The residuals for all three models are extremely similar and, despite some evidence of a nonrandom pattern, show no significant serial correlation at lags <10 yrs (Fig. 4). Statistical evidence for any form of biological interaction is therefore weak at best, as all three models fit the data about equally well. However, as our model has 2 fewer parameters than the Larkin model, it does appear to provide a better description of possible between-year density-dependent interactions in Adams River sockeye, and we concentrate on comparing the predictions of this model with those of the Ricker model in subsequent sections.

Parameter Uncertainty

Uncertainty in parameter estimates for nonlinear models is usually quantified by assuming that the model is at least locally linear, and that the standard statistical formulae appropriate to linear regressions are applicable. However, decomposition of the nonlinearity for the Adams River model-data combination indicates that the usual linearization approach would provide inaccurate confidence regions about the parameter estimates for the generalized model (Table 2A). A likelihood-based assessment of the uncertainty in the estimated parameters is therefore required for the generalized model.

A confidence region constructed using the likelihood method is determined by finding those parameter estimates that satisfy,

$$(18) \quad S(\alpha, \beta, \rho) - S(\alpha^*, \beta^*, \rho^*) \leq p s^2 F_{p,N-p,0.95}$$

in situations fitting the NIID assumptions (Donaldson and Schnabel 1987; Welch and McFarlane 1990). Here, $S(\alpha, \beta, \rho)$

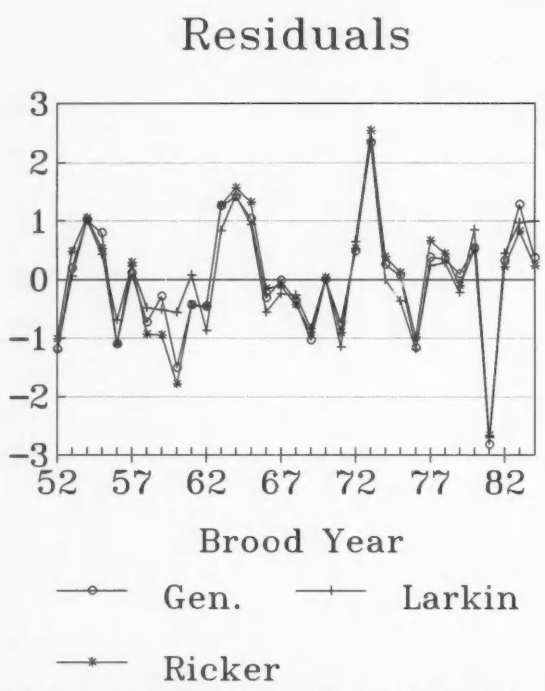

FIG. 4. Residual plots for the three recruitment models. The first 4-yr of data (1948–51) were not used in the fitting process except as estimates of lagged stock.

is the residual sum of squares obtained using the parameters (α, β, ρ); $p = 3$ is the number of parameters fitted, and s^2 is the residual variance. In words, this describes a region about the maximum likelihood parameter estimates, α^*, β^*, ρ^*, that includes all alternative parameter estimates lying within the stated difference in residual sum of squared errors. This region is likely to contain the true parameter values with 95% probability (Donaldson and Schnabel, 1987). Calculation of the joint 95% confidence region about the initial maximum likelihood estimates for the Adams River data shows that it is extremely broad (Fig. 5), and includes the null hypothesis of $\rho = 0$ as well as the bias-corrected parameter estimates (see below). It is therefore impossible to reject the possibility that no biological interaction occurs between runs returning to the Adams River, despite the magnitude and persistence of the historical cycle.

Bias

Parameter estimates for nonlinear models may be biased for at least two reasons: fundamental nonlinearity of the model and objective surface (Bates and Watts, 1980; Ratkowsky, 1983), and time series correlations between the environmental disturbances and future stock sizes (Box and Jenkins 1976, p.412; Walters, 1985). Application of the analysis advocated by Box (1971) and Bates and Watts (1980) suggests that bias is unlikely because of the intrinsic nonlinearity of the model, but these diagnostics say nothing about the bias due to time series errors caused by correlation between environmental influences and future stock sizes.

An as yet unpublished result by Walters (Resource Ecology, University of British Columbia, Vancouver, B.C. V6T 1W5, pers. comm.,) shows that to a good approximation an unbiased estimate of the density-dependent parameter β can be calculated as:

$$(19)\quad \beta_{TRUE} = \hat{\beta} - \frac{\bar{S}\sigma_\epsilon^2(1 + 2\Theta_\epsilon)(n_c - 1)}{2SS_X},$$

where $\hat{\beta}$ is the initial (biased) estimate of the density-dependent parameter, $\bar{S} = 251\,901$ is the estimate of mean stock size across all cycles, $\sigma_\epsilon^2 = 1.09$ is the residual variance, $\Theta_\epsilon = 0.17$ is the estimated magnitude of the noise autoregressive term (i.e. the serial correlation of the noise at lag one), n_c are the number of cycles observed (eight), and SS_X is the sum of squared stock sizes (8.61×10^{12}).

We have not attempted to calculate analytic correction factors for α and ρ owing to the great uncertainty in the value of ρ, and the small size of the bias correction factor (15%) predicted by Walters' equation (19). Instead, we took the bias corrected estimate of β, and calculated the maximum likelihood estimates of α and ρ conditional on this value. The result (Fig. 5;

TABLE 2. (A) Calculation of the maximum curvature measures (Bates and Watts 1980) gives an indication as to whether linearization techniques can be used to evaluate uncertainty in parameter estimates from nonlinear models. When either the intrinsic curvature, Γ_{IN}, or the parameter-effects curvature, Γ_{PE}, is greater than the critical value $1/(2\sqrt{F_{p,N-p,0.95}})$, calculation of confidence regions by linearization (the usual method) is inappropriate, and likelihood-based assessments of the uncertainty are necessary. The table shows scaled maximum curvature estimates before and after applying Walters' correction (equation 19) for the time series bias. Values >1 indicate significant nonlinearity. (B) Parameter estimates for the Ricker and generalized models before and after applying Walters' correction to remove the time series bias. "% bias" is the predicted bias due to distortion caused by nonlinearity of the model equation, measured as a percent of the estimated value. See Box (1971) or Ratkowsky (1983) for details of this calculation.

A. Maximum curvature measures

	Ricker		Generalized	
	Before	After	Before	After
$\frac{\Gamma_{IN}}{2\sqrt{F_{p,N-p,0.95}}}$	0.0	0.00	1.90[a]	0.65
$\frac{\Gamma_{PE}}{2\sqrt{F_{p,N-p,0.95}}}$	0.0	0.00	5.51[a]	5.88[a]

B. Estimated bias

	Ricker			
	Before		After	
Parameter	Estimate	% bias	Estimate	% bias
α	11.82	0.00	11.44	0.00
β	0.77×10^{-6}	0.00	0.65×10^{-6}	0.00
σ_ϵ^2	1.12		1.12	
	Generalized			
α	19.17	0.49	20.1	0.61
β	1.07×10^{-6}	−0.32	0.90×10^{-6}	0.00
ρ	0.63	−0.70	0.71	0.55
σ_ϵ^2	1.09		1.11	

[a]Indicates significant nonlinearity.

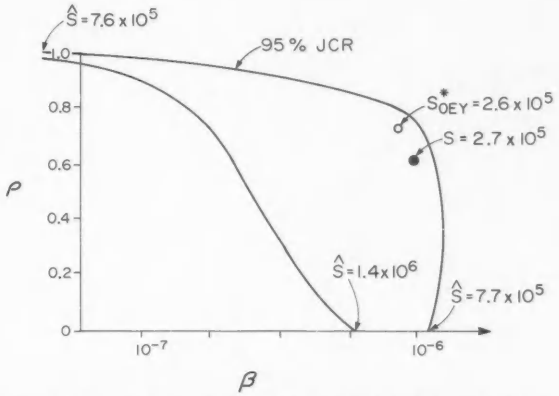

FIG. 5. Maximum likelihood (ML) estimates of the parameters β, ρ calculated from the generalized model, and the 95% joint confidence region calculated from equation (18). The confidence region includes the null hypothesis of no between-year density-dependent interaction ($\rho = 0$). Optimal stock sizes, S, corresponding to the ML (●) and bias-corrected (○) parameter estimates are shown (assuming an equal escapement policy) along with estimated stock sizes at several points on the periphery of the confidence region, $\hat{S}$. Note that although the confidence region extends far to the left of the graph, the estimate of optimal stock size remains quite constant.

Table 2B) still lies well within the 95% joint confidence region about the original parameter estimates.

Optimal Escapement Strategies for the Generalized Model.

We now compare the effect of imposing different escapement patterns on the Adams River sockeye returns, assuming the generalized model to be correct, and then contrast the estimates of optimal stock size and yield with those for the Ricker model. Although the uncertainty in the parameter estimates for the generalized model includes the possibility of no interaction, the point estimates suggest rather powerful interaction may occur. Contrasting these two models therefore gives a good idea of what the influence of between-year interactions on potential yields may be.

Under a lognormal error distribution, mean recruitment exceeds modal recruitment by a factor of $\exp(\sigma^2/2)$. This implies arithmetic mean recruitment yields will be 72% (assuming $\sigma^2 = 1.09$) greater than the modal estimates calculated from the deterministic equation for the generalized model. A similar argument applies to the estimated yields from the Ricker model, but here the inflation factor is $\exp(1.12/2) \approx 1.75$. We have included the appropriate expansion factor in the calculation of expected yield for both models and all escapement policies described below.

If we accept that between-year density-dependence may occur in the Adams population, what is the optimal management strategy? As we have noted, one optimum is obtained by holding escapement fixed over all 4 yr of the cycle. If density-dependence is high enough, it seems feasible that allowing the Adams River to lie fallow for one or more years might improve the total yield. This is a tactic commonly followed in agriculture. The procedure is also similar to the current cyclic pattern of returns, since in 2 yr of the cycle the spawning areas in the Adams are essentially unused.

Estimates of optimal stocking density under several imposed escapement patterns show that, as expected, forcing escapement to zero in one or more years of the cycle increases the optimal stocking densities for the remaining years of the cycle (Fig. 6), since the system has more time to recover before escapement is again allowed. Forcing the escapement to match either the alternating or extreme cyclic patterns results in the nonzero escapement levels rising to a level about 60% greater than the equal escapement strategy. However, total escapement summed over the 4 yr of the cycle does not vary greatly, ranging from a low of 750 000 for the current pattern, to a high of 1 million for the equal escapement policy.

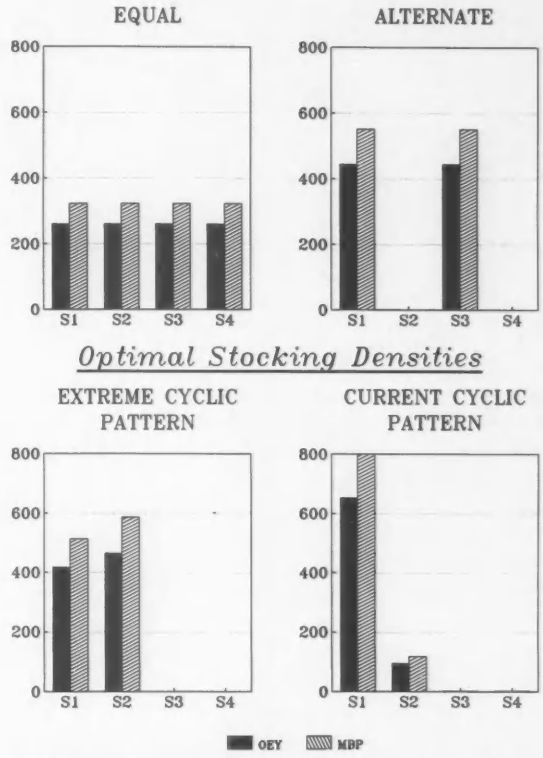

FIG. 6. Optimal escapement levels calculated for the four recruitment patterns considered in the text. Escapement is in thousands of effective females. Stock sizes achieving optimal economic yield (*OEY*) and maximum biological production (*MBP*) are shown for each recruitment pattern. S1–S4 = cycle years.

Choosing the "extreme" escapement policy of reducing two consecutive runs to zero also results in reversing the dominance pattern from its historic pattern. These findings remain consistent if one were to (incorrectly) manage to maximize the maximum biological production (MBP) from the system, rather than to maximize the harvestable surplus (OEY). Surprisingly, we found that the optimal stocking densities are similar to current escapement levels if we constrain the optimization to maintain the current cyclical pattern while optimizing only the magnitude of the cycle (Appendix B). We comment on this point in greater detail below.

Despite the potential benefits of allowing the system to lie fallow, an equal escapement policy still gives maximum yields over the 4-yr-cycle for the generalized model (Fig. 7). Both the "alternate" and "extreme" patterns lead to essentially identical production, but with yields almost 2 million fish per cycle

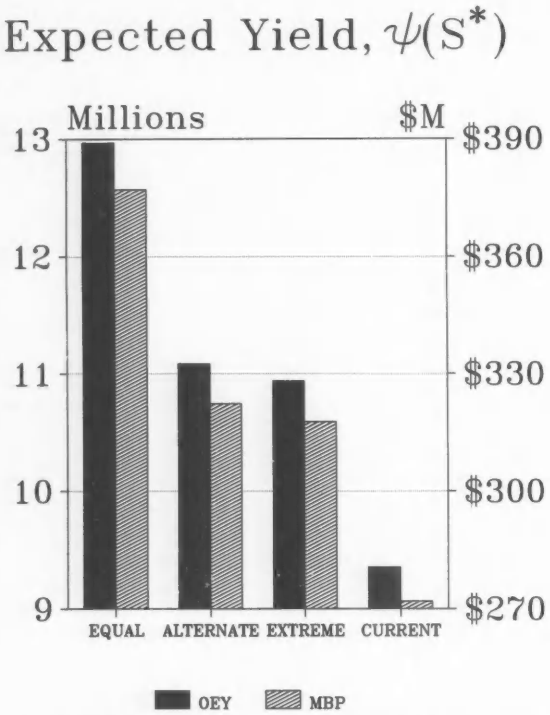

FIG. 7. Optimal economic yield per cycle for the four escapement strategies considered in the text. Yield estimates are increased by the factor $\exp(\sigma_\epsilon^2/2) \approx 1.7$ to convert from modal to expected mean recruitment in both this and the next figure.

below the equal escapement policy. Maintaining the current cyclic pattern, but at the optimal escapement level, resulted in the worst yields of all, with yields 3.5 million fish per cycle below the optimum.

Optimal Yield Estimates: Ricker versus Generalized Model.

At the current economic return of ca. $30 per fish, a difference of 3–5 million fish per cycle is very large. Even ignoring the possibility that the Ricker model may be correct, the different escapement policies represent a potential gain relative to the current cyclical pattern of returns of up to $100 million per cycle.

The difference in optimal yields under the various management regimes is even greater if between-year density-dependence is not present, and the Ricker model is correct. Estimates of optimal stock size and yield for the generalized model are substantially smaller than those for the Ricker model (Fig. 8), reflecting the effect of significant negative interaction between the runs predicted by the large interaction coefficient ($\rho = 0.71$). Assuming constant escapement, the optimal economic yield over the 4-yr-cycle for the generalized recruitment model is 13 million fish, achieved at an escapement of 260 000 effective females per annum. Note that the effect of a large ρ in the generalized model is to flatten the dome of the stock–recruitment relationship, so that the stock sizes and yields obtained at the OEY and MBP points become quite similar.

Our estimates of optimal escapement and yield for the Ricker model (1.1 million effective females, achieving a 4-yr-optimal

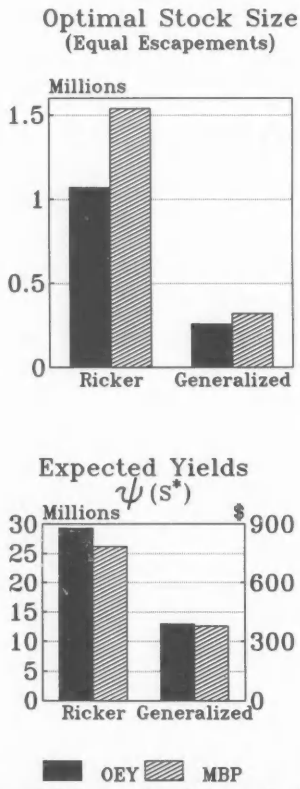

FIG. 8 (*Upper panel*) Estimates of stock size required to achieve optimal economic yield (*OEY*) and maximum biological production (*MBP*) for the Ricker and generalized stock–recruitment models. The economic optimum stock size for the generalized model is 24% of the Ricker model estimate. (*Lower panel*) Optimal yield estimates (4-yr-total) for the Ricker and generalized recruitment models. Total recruitment yield for the generalized model is 44% of the estimated yield for the Ricker model. Note that for high levels of between-year interaction the difference in yield between the *MBP* and *OEY* policies becomes negligible.

yield of 29.3 million recruits), are similar to estimates obtained by Collie and Walters (1987) for the Ricker curve. The estimates of escapement and recruitment from the generalized recruitment model are therefore only 25 and 50%, respectively, of the values predicted by the Ricker model. Since a catch of 1 million sockeye is worth ca. $30 million, density-dependent interaction seriously reduces potential economic yield.

Discussion and Conclusions

Cyclic dominance in Pacific salmon seems to be like pornography: lacking clear technical definition, but widespread and quickly identified by the casual observer. The only clear definition of this term we have found is from Ward and Larkin (1964, p.17): "The most important feature of well-developed dominance is the great, consistent disparity between dominant and off-year populations." The term has been applied (frequently uncritically) to apparently cyclical patterns in the sockeye returns to other stocks within the Fraser River, to sockeye returns to the Skeena River (Larkin and McDonald 1968) and

Bristol Bay (Eggers and Rogers 1987), and to the striking even or odd year pink salmon runs. It is still unclear whether cyclic "dominance" is merely a pattern in returns, or whether it indicates the existence of hidden biological mechanisms controlling and shaping the cycle.

In a nutshell, our results indicate that there is only weak statistical support for the existence of between-year density-dependent interactions in the Adams River sockeye, and the uncertainty in the estimates is so large that it easily includes the alternative hypothesis of no between-year interaction at all. Statistical analysis of the historical data therefore has little to tell us about the mechanisms regulating the Adams River sockeye. The inability to identify an effect of past spawning levels on recruitment has important implications for the management of the population.

It seems obvious in retrospect from the sheer amplitude of the Adams River recruitment cycle that extremely strong interaction would be needed before detection could be possible. Because the Adams run comigrates with other late-run Fraser stocks, the Adams stock makes up only 2–3% of the total sockeye catch in weak years. Large classification error probably creeps into the assignment of catch to the Adams River stock for these years, although the estimation of run size for the two strong years of the cycle is likely more reasonable. Even ignoring classification errors, the per capita influence of off-year fish would have to be 500 times that of the dominant if a measurable effect of variation in escapement in the weak years on the strong year's recruitment was to be found. Similarly, the large errors in the recruitment estimates for the off years means that there may be little signal present to correlate with the escapement levels in the subdominant and dominant years that follow.

There is therefore a fundamental asymmetry about the Adams River cycle: small runs are almost certainly too small to have significant biological influence on the dominant run, while they in turn are so small that any response of their recruitment patterns to fluctuations in escapement may well be statistically undetectable because of measurement errors. A strong case can therefore be made for an adaptive management approach involving the rebuilding and equalizing of runs to each year of the cycle. We have been able to show that even if interaction does exist, an equal escapement policy should bring substantial economic benefits. Attempts to rebuild this stock should also yield important biological information about the existence of interaction, and help to distinguish biological fact from speculative fiction.

Our proposed stock–recruitment model provides at best only a marginally better description of the Adams River sockeye dynamics than either the Ricker or the Larkin models. Because of the fairly simple mathematical form of this model, we have, however, been able to analytically derive the relative productivity of the stock under different management regimes. Because the equations are nonlinear, we cannot unequivocally prove that better alternatives to an equal escapement strategy do not exist when between-year interaction occurs. We have been able to demonstrate that the constant returns scenario is at least a local optimum, and our numerical investigations suggest that this strategy is almost certainly globally optimal. This result agrees with the numerical investigations reported by Collie and Walters (1987) for the Larkin model.

The issue of cyclic dominance is potentially of much wider applicability than to just the Adams River sockeye, or even to the other stocks of Fraser R. sockeye. The same arguments may also apply to cyclic dominance in the Skeena (Larkin and McDonald 1968) or Alaskan sockeye (Eggers and Rogers, 1987), or indeed to the striking odd year dominance patterns of pink salmon in the Fraser River.

We have deliberately made the analogy with agriculture at several points, because the implicit assumption that seems to lie behind some biological interpretations of cyclic dominance is that the gains from increasing stock densities in the weak years of the cycle cannot make up for the loss in production from the dominant run. These arguments have exact analogues in agriculture: if crops are planted too densely, total yields go down. Failing to let fields occasionally lie fallow, with little or no production, leads to the same result.

We have been able to show that such scenarios are not implicit in the model we have presented and fitted to the Adams River data. Neither variations on the current cyclical pattern, nor the imposition of an escapement policy where no escapement is allowed in alternate years (a possibility suggested by Ricker 1950), can match the production potential of setting escapement to all four runs equal. Given the uncertainty in the data, and the almost identical fits found for the Ricker, Larkin, and generalized models, it seems unlikely that a more statistically satisfying model of interaction in the Adams River stock is likely to emerge in the short term. In particular, the equivocal performance of Larkin's model, despite its greater flexibility, indicates that the possibility of depensatory suppression of weak runs by a lagged predator response is not supported by the data.

Previous efforts at managing the stock without upsetting the current cyclical pattern therefore need some careful re-evaluation. The strong return of sockeye to the Adams River in 1971 provides a case in point, when the subdominant run threatened to reverse the historic dominance pattern by returning in greater strength than the previous year's dominant run. The run was deliberately subjected to an extremely high harvest rate (91%) in order to reduce the escapement to historic levels and to satisfy "...the relationships that appear to have existed in Shuswap Lake for many years between plankton, predators and the production and survival of young sockeye," despite the severe effect this had on comigrating stocks (IPSFC 1972; p. 25).

It is somewhat ironic that we find reversing the dominance pattern, while not optimal, probably would have substantially increased the total yield from the Adams. Contemporary studies (Larkin 1971) also suggested that a reversal of the dominance pattern would have been stable.

Such post hoc analyses of past events of course ignore the difficulty of making such decisions in, at best, a matter of days, as has been the Pacific Salmon Commission's lot. They do, however, suggest that an explicit attempt to both increase and equalize the 4 runs to the system would have significant economic payoffs, in addition to stabilizing the catches and providing important information about the level of biological interaction that really does exist.

The magnitude of these benefits are potentially staggering: even assuming that interaction as high as that estimated by the generalized model does exist ($\rho = 0.7$), an equal escapement policy could increase yields by at least 35%. If an equal escapement policy was instantly achievable, at current value-added prices this is equivalent to an additional $27 million per annum, or an increase in net present value assuming a 4% discount rate of $675 million from the Adams stock alone. If interaction between the runs does not exist, then the potential benefits are huge: on the order of $3.7 billion.

These calculations have a large element of the quixotic about them; they disregard the need to build up the weak runs before

reaping such windfalls, and they ignore the inevitable emergence of new bottlenecks surely intended to violate the simple assumptions lurking behind our mathematics. Nevertheless, even if other forms of density-dependent regulation were to develop, calculations such as those above suggest a change in management strategy should still prove highly profitable.

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Appendix A: Maximum Biological Production

Case I: Derivation of the equal stock size solution

Making the assumption that one solution to equation (6) is $S_i = S_{MBP}$, $i = 1,..,4$, then

$$\text{(A1)}\quad \frac{\partial\psi(S)}{\partial S_j} = \alpha \exp\left\{\frac{-\beta}{1-\rho^4}\sum_{t=0}^{3}\rho^t S_{MBP}\right\} \times \left\{1 - \frac{\beta}{1-\rho^4}\sum_{i=1}^{4} S_{MBP}\rho^{[i-j]}\right\}$$

must equal zero. Therefore, since the first term is always >0 for biologically realistic parameter values ($0 \leq \beta$, $S_{MBP} < \infty$ and $0 \leq \rho < 1$),

$$\text{(A2)}\quad 1 - \frac{\beta}{1-\rho^4}\sum_{i=1}^{4} S_{MBP}\rho^{[i-j]} = 0,$$

so,

$$\text{(A3)}\quad S_{MBP}\{1 + \rho + \rho^2 + \rho^3\} = \frac{1-\rho^4}{\beta},$$

which yields

$$\text{(A4)}\quad S_{MBP} = \frac{1-\rho}{\beta}.$$

Case II: pink salmon life history

Setting $S_2 = S_4 = 0$ and taking the derivative w.r.t. S_1,

$$\text{(A5)}\quad \frac{\partial\psi(S)}{\partial S_1} = \alpha\left(\exp\left\{\frac{-\beta}{1-\rho^4}\sum_{t=0}^{3}\rho^t S_{[1-t]}\right\} - \frac{\beta}{1-\rho^4}\sum_{i=1}^{4} S_i\rho^{[i-1]}\exp\left\{\frac{-\beta}{1-\rho^4}\sum_{t=0}^{3}\rho^t S_{[i-t]}\right\}\right)$$

$$\text{(A6)}\quad = \alpha\left[\exp\left\{\frac{-\beta}{1-\rho^4}(S_1 + \rho^2 S_3)\right\} - \frac{\beta}{1-\rho^4}\left\{S_1 \times \exp\left\{\frac{-\beta}{1-\rho^4}(S_1 + \rho^2 S_3)\right\} + \rho^2 S_3 \exp\left\{\frac{-\beta}{1-\rho^4}(S_3 + \rho^2 S_1)\right\}\right\}\right]$$

so,

$$\text{(A7)}\quad \exp\left\{\frac{-\beta}{1-\rho^4}(S_1 + \rho^2 S_3)\right\} = \frac{\beta}{1-\rho^4}\left[S_1 \exp\left\{\frac{-\beta}{1-\rho^4} \times (S_1 + \rho^2 S_3)\right\} + \rho^2 S_3 \exp\left\{\frac{-\beta}{1-\rho^4}(S_3 + \rho^2 S_1)\right\}\right]$$

which may be rearranged to yield

$$\text{(A8)}\quad \frac{1-\rho^4}{\beta} = S_1 + \rho^2 S_3 \exp\left\{\frac{-\beta}{1-\rho^4}((1-\rho^2)S_3 + (\rho^2-1)S_1)\right\}$$

A similar result holds after taking the derivative w.r.t. S_3:

$$\text{(A9)}\quad \frac{1-\rho^4}{\beta} = S_3 + \rho^2 S_1 \exp\left\{\frac{-\beta}{1-\rho^4}((1-\rho^2)S_1 + (\rho^2-1)S_3)\right\}$$

By inspection, these two expressions are equal if and only if $S_1 = S_3 = S_{MBP}$, leading to

$$\text{(A10)}\quad S_{MBP} = \frac{1-\rho^2}{\beta}.$$

Case III: extreme cyclic pattern

Setting both S_3 and S_4 to zero, we take the derivative w.r.t. both S_1 and S_2 and solve for the stock sizes giving maximum recruitment in a procedure identical to the previous cases. Since both equations must equal zero at a maximum, this implies that:

$$(A11)\quad S_1 + \rho S_2 \exp\left\{\frac{-\beta}{1-\rho^4}[(\rho-1)S_1 + (1-\rho^3)S_2]\right\} = S_2 + \rho^3 S_1 \exp\left\{\frac{-\beta}{1-\rho^4}[(1-\rho)S_1 + (\rho^3-1)S_2]\right\}.$$

Although we have not found an explicit analytical expression for the two stock sizes, it is possible to show that their ratio must satisfy:

$$(A12)\quad \frac{S_1}{S_2} = \frac{1-\rho\exp\left\{\frac{\beta}{1-\rho^4}[(1-\rho)S_1+(\rho^3-1)S_2)]\right\}}{1-\rho^3\exp\left\{\frac{-\beta}{1-\rho^4}[(1-\rho)S_1+(\rho^3-1)S_2)]\right\}}.$$

Taking the example $\beta=1$, $\rho=0.5$ for illustration, we find that $S_1<S_2$, with $S_1\approx0.67$, and $S_2\approx0.82$, the opposite of the dominant:subdominant pattern historically seen in the Adams River sockeye returns. The pattern obtained using the maximum likelihood parameter estimates reported in the text is similar.

Appendix B: Optimal Economic Yield

In Appendix A we derived expressions for the stock size required to obtain maximum biological production, S_{MBP}, and the biological production that resulted. This is useful chiefly to show how the parameters of our generalized model influence productivity. In fact, the harvestable surplus is maximized at smaller stock sizes. We now derive the equation that defines the (economically) optimal stock size, S^*_{OEY}, and the 4-yr-yield from the system under this scenario.

Surplus production is defined as the recruitment remaining after allowing sufficient escapement to just maintain the population. Maximizing this quantity leads to the optimal economic yield. Defining the surplus recruitment as

$$(B1)\quad Y(S) = R(S) - R_{rep}(S),$$

i.e. the difference between actual recruitment and that required to just replace the population, then our goal is to find the stock size that maximizes the harvestable surplus, S^*_{OEY}.

We chose to scale the abundance of the population in the i^{th} yr of the cycle as $S_i=\gamma_i S$. From geometry, it is easy to show for a population whose recruits all mature at the same age that the level of recruitment needed to just replace the population is:

$$(B2)\quad R_{rep}(\gamma_i S) = \alpha\gamma_i S \exp\left\{-\beta\sum_{t=0}^{\infty}\rho^t\gamma_{[i-t]}S_{EQ}\right\},$$

where S_{EQ} is the unfished equilibrium stock size. Substituting text equation (2) for our generalized model and equation (B2) into (B1), the harvestable surplus over the 4-yr-cycle is:

$$(B3)\quad \psi(S) = \sum_{i=1}^{4} R(\gamma_i S) - R_{rep}(\gamma_i S),$$
$$= \alpha S\sum_{i=1}^{4}\gamma_i\left(\exp\left\{\frac{-\beta S}{1-\rho^4}\sum_{t=0}^{3}\rho^t\gamma_{[i-t]}\right\} - \exp\left\{\frac{-\beta S_{EQ}}{1-\rho^4}\sum_{t=0}^{3}\rho^t\gamma_{[i-t]}\right\}\right).$$

The ratio of recruits to spawners at the unfished equilibrium can be defined as $\zeta = R(\gamma_i S_{EQ})/\gamma_i S_{EQ}$. (If both recruits and spawners are measured in the same units then $\zeta=1$, else $\zeta\neq1$)[1]. Therefore,

$$(B4)\quad \zeta = \alpha\exp\left\{\frac{-\beta S_{EQ}}{1-\rho^4}\sum_{t=0}^{3}\rho^t\gamma_{[i-t]}\right\}.$$

Taking logs and solving for S_{EQ},

$$(B5)\quad S_{EQ} = \frac{(\ln(\alpha) - \ln(\zeta))(1-\rho^4)}{\beta\sum_{t=0}^{3}\rho^t\gamma_{[i-t]}}.$$

Finally, substituting this expression into equation (B3) gives us our expression for the optimal economic yield:

$$(B6)\quad \psi(S) = \alpha S\sum_{i=1}^{4}\gamma_i\left(\exp\left\{\frac{-\beta S}{1-\rho^4}\sum_{t=0}^{3}\rho^t\gamma_{[i-t]}\right\} - \frac{\zeta}{\alpha}\right)$$

Equation (B6) can be used in either of two ways. First, we can specify a given recruitment pattern γ_i (for example, the historic ratio of mean returns to the 4-yr of the cycle, 474:69:1.5:1) and then find numerically the value of S that maximizes the 4-yr-yield, S^*_{OEY}. The values $S^*_{i,OEY}=\gamma_i S^*$ give the stock size for the i^{th} year of the cycle that achieves the economically optimal yield, while maintaining the specified return pattern. We used this approach to calculate the optimal yield under the constraint that the cyclic pattern of the returns was not changed. Alternatively, we can find the optimal yield without this constraint by maximizing (B6) directly with respect to the parameters γ_i and setting $S=1$. Constraining one or more of the γ_i to have specific values allows assessment of the economically optimal stock sizes for specific life history patterns. For example, optimal stock sizes for a pink salmon life history are found by setting $\gamma_2=\gamma_4=0$, and maximizing with respect to γ_1 and γ_3.

[1]Collie and Walters (1987; p.1556) report that from 1948–84 59.4% of returns were female, with a mean spawning success of 96.6%. This leads to $\zeta=1.74$.

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